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ÐME EXPERIMENTS ON THE NATURE AND FUNCTION OF REISSNER'S FIBER

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THIRTY-FIVE FIGURES

CONTENTS

It is probable that concerning no part of the vertebrate nervous system have there been held views more widely divergent than those which have been entertained concerning Reissner's fiber.

In 1907, when I took up the study of this structure, Sargent's 'optic reflex' theory had met with very general acceptance. At an early stage in my work, however, I obtained proof that the

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nervous system. Accordingly, there will be need, at this time, only for a brief review of the various suggestions which have been put forward as to the nature and function of the fiber and a short account of the present state of our knowledge of the fiber and its connections, the reader being referred to the above mentioned work for further details.

I gladly avail myself of this opportunity to express my thanks to Professor Dendy for valuable advice and criticism throughout the progress of the work: also, to the Government Grant Committee of the Royal Society, for Grants in Aid; to the British Association for the Advancement of Science and the Senate of the University of London for placing at my disposal their tables at the Plymouth Marine Laboratory, and to Dr. Allen, Director of the Laboratory, for the facilities afforded me in the prosecution of the research.

I. INTRODUCTION

A. A review of the suggestions which have been made concerning the nature and function of Reissner's fiber and the sub-commissural organ

1. Reissner ('60), by whom the fiber which now bears his name was discovered, believed that this 'Centralfaden' was simply a nerve fiber and to him, therefore, it was remarkable principally on account of its peculiar situation. He found it, as is well known, lying freely as an axial thread in the central canal of the spinal cord of the lamprey. Since the diameter of the fiber in this animal (in which alone he had observed it) is, approximately, that of a moderately coarse nerve fiber, it is scarcely surprising that, its unusual situation notwithstanding, Reissner came to this conclusion. Kutschin ('63) who confirmed Reissner's discovery, accepted that author's view of its nature. Neither of these observers was able to trace the fiber into the brain ventricles and they believed it to be confined to the central canal of the spinal cord.

2. That a nerve fiber should occur in such a situation seemed to Stieda ('68, '73) altogether improbable and he decided that Reissner's fiber ('jenen räthselhaften Strang') must be an arti-

fact. He suggested that the alleged fiber was produced by the coagulation of the cerebro-spinal fluid under the action of the fixing reagent, pointing out that there was no evidence of its being related to any nerve cell.

For thirty years this view passed almost unquestioned, Viault ('76), Rohon ('77), Sanders ('78, '94) and Gadow ('91) all accepting it. More recently Kalberlah ('00), Streeter ('03) and Edinger ('08) have expressed themselves in agreement with Stieda's view. That this view was so widely held is, doubtless, the explanation of the fact that during this period there are found, in the literature, so few references to the occurrence of the fiber.

3. Interest in this structure revived, however, when Studnička ('99) reasserted the preformed nature of the fiber. This author suggested that it was to be regarded as an epithelial secretion, comparable to that which has produced the crystalline style of the lamellibranch gut. He believed that it is produced by the cells lining the central canal of the spinal cord and that it is capable of growing forward, to end freely in the brain ventricles but he made no suggestion as to its function. Kolmer ('05) appears to be the only author who has endorsed this view and Studnička has, himself, since abandoned it ('13).

4. It is a very surprising fact that the extraordinary and quite conspicuous development of the epithelium beneath the posterior commissure, should have remained for so long unnoticed. A brief mention of it, indeed, appears to have been made by Fulliquet ('86) but not until 1892 was it figured (very diagrammatically) by Edinger ('92) who conjectured that it might be a glandular body producing some secretion to be discharged into the cerebro-spinal fluid. Its histology was first carefully described by Studnička ('00) who gave figures of its finer anatomy in dogfish and lamprey but did not, apparently, realize its connection with Reissner's fiber.

5. A little later the sub-commissural organ of the Ammocoete was described and figured by Dendy ('02) who noted the existence of close-set cilia clothing its ventricular surface and suggested that, in conjunction with certain folds of the choroid plexus

of the midbrain, it served to establish currents which promoted the circulation of the encephalic fluid.

6. In the meanwhile Sargent had also asserted the preformed nature of Reissner's fiber but had denied that Studnička was correct in interpreting it as a secretion. In Sargent's view the fiber was a nervous structure.

In several subsequent papers ('01, '03, '04) Sargent endeavored to establish this view stating that Reissner's fiber consists of "numerous axis cylinders closely applied to each other and surrounded by a single thin medullary sheath of myelin." These axis cylinders were supposed to be derived in part from the numerous large cells of the 'Dachkern' and from alleged multipolar cells in the habenular ganglion as well as from other multipolar cells said to be situated actually within the lumen of the central canal, towards the hinder end of the spinal cord. In teleosts, in which group Sargent overlooked the remnants of the 'Dachkern,' he claimed that the alleged midbrain constituent "axons" of Reissner's fiber were derived from the myriad cells of the torus longitudinalis.

Reissner's fiber was, therefore, according to this author, built up of two sets of axons running in opposite directions and a comparison was made between this structure and the giant fibers of Amphioxus and Annelida. Concerning the destination of the forwardly running axons there is nothing stated, but those which were said to arise in the brain were regarded as motor axons having a very great length, each being supposed to stretch from the midbrain roof direct to one of the trunk muscles. Sargent stated that he had seen such fibers leaving the main Reissner's fiber in the region of the spinal cord and that these passed out directly to the musculature, probably by way of the ventral spinal roots. In the midbrain roof the related nerve cells were described as in direct connection with the proximal ending of the retinal neurons so that there was said to be interposed but a single nerve element between the sensory (retinal) nerve cell and the muscle-fiber in the trunk. Sargent suggested that, by this means, the delay in the transmission of motor stimuli along

the ordinary (tecto-spinal) conduction paths through a number of neurons could be lessened in cases of urgency.

Houser ('01) claimed that he had been able to confirm Sargent's observations, while numerous observers seem to have accepted Sargent's theory concerning the function of the fiber.

That, notwithstanding many weighty objections, this theory met with such general acceptance is doubtless to be attributed very largely to the fact that Sargent claimed ('04) that his observations had been fully confirmed by actual experiments upon living animals (vide infra).

7. Although Sargent ('03) was the first to describe the connection between Reissner's fiber and the sub-commissural organ (his 'ependymal groove') he attributed comparatively little importance to this latter structure, asserting that it served merely as a support and anchorage for Reissner's fiber. In this view he has been followed recently by Tretjakoff ('13).

Kölliker ('02) recording the occurrence of Reissner's fiber in the blind Proteus and other Amphibia, admitted that he had become convinced of the preformed nature of the fiber. He appears, however, to have been unable to choose between the conflicting views advanced by Studnička, Sargent and Kalberlah.

8. The work of Ayers upon 'Ventricular Fibers in Myxinoids' is of interest in that it contains the first suggestion that Reissner's fibers might be composed of numerous united delicate fibrillae springing from ependymal epithelial cells. Whether, however, he considers these fibrillae as of the same nature as the ependymal fibers which serve as supporting structures within the central nervous system, or not, Ayers does not make clear, and his work unfortunately contains a number of erroneous statements. He does not, indeed, refer to the fiber by name and appears to have been wholly unaware of previous work upon the subject.

Thus, in Bdellostoma, he figures numerous more or less parallel ventricular fibers which, while they may perhaps represent several lengths of a much folded and snarled fiber, may equally well represent some artifact. It certainly is not the normal condition in this animal. Moreover, it would appear that

Ayers never saw Reissner's fiber in the lamprey, since his description of the 'ventricular fibers' in that animal as "a fine-meshed network of fibrils which . . . . in life practically fills the ventricular cavity" certainly can not apply to Reissner's fibers. It is extremely probable, therefore, that Ayers failed to distinguish clearly between coagulum and the fibrillae of Reissner's fiber. His conclusion that the fiber was certainly "an organ of relation bringing *all parts* of the ventricular cavity into intimate connection" (my italics) is likewise mistaken, for Ayers did not correctly identify the brain cavities in this animal, in which of the iter little remains but the sub-commissural canal. Accordingly he failed to recognize the distinction which exists between the tract of modified epithelium which constitutes the sub-commissural organ and the flattened epithelium which lines other parts of the ventricular cavity. Concerning the function of the fiber he conjectured that it might be "connected with the control of the ventricular lymph supply by vaso-motor control."

9. Horsley ('08) describing the occurrence of Reissner's fiber in certain apes stated that, in these forms at least, the fiber had not the structure of a tract of nerve fibers nor, when cut, did it exhibit Wallerian degeneration. While not denying the accuracy of Sargent's statements in so far as they relate to this structure in the lower vertebrates, Horsley expressed the opinion that, in its resiliency, the fiber resembled a chitinous or skeletal structure and suggested that, in the higher vertebrates, it had become nothing more, perhaps, than a residual structure.

10. In 1909 I gave an account ('09) of the behavior of the fiber in recoil and stated that, in my opinion, the fiber was certainly non-nervous. At the same time Dendy ('09) put forward an entirely novel suggestion concerning the function of the fiber. His suggestion was that the fiber itself was a strand of connective tissue which played a merely mechanical part, variations in its tension being produced by the flexure of the body and every such variation might be supposed to result in a stimulus being transmitted to the cells of the sub-commissural organ This latter structure was interpreted as a sensory

organ, controlling automatically the flexure of the body. He concluded by expressing the hope that some way would be found of overcoming the apparently insuperable obstacles which stood in the way of satisfactory experiments upon the fiber by which alone could the hypothesis be tested.

11. A study of the development of Reissner's fiber in Cyclostomes (and Amphibia) led me to the conclusion ('12, '12a, '13) that this structure was formed by the coalescence of cilia-like processes springing from cells which, while largely collected upon the sub-commissural organ, are not limited to that organ, other cells occurring scattered in the ependymal lining of the central canal contributing to the fiber. In my opinion, the fiber is to be regarded as a thread of protoplasm. This view is supported by the staining reactions of the fiber, while its high refractivity, its power of regeneration and the rapidity with which it apparently disintegrates after death are facts easily explicable upon this hypothesis. Further, its mode of contraction is paralleled, only, so far as I am aware, in the scarcely modified protoplasm which forms the stalk of certain Protozoa.

This view that the fiber is, in fact, a protoplasmic thread has since been accepted by Dendy ('12), Studnička ('13) and by Tretjakoff ('13). The latter author, however, appears to have misread Sargent's papers, for he attributes this view to that investigator, saying ('13, p. 110) "Sargent zeigte nämlich, dass der Faden noch in embryonalen oder larvalen Leben als ein Bundel von feinen, cilienahnlichen Fortsätzen der Zellen der Sub-kommissuralen Grube ensteht."

12. Tretjakoff ('13), however, while accepting this view of the nature and function of the fiber suggests that we are mistaken ("ich glaube deswegen, dass in diesem Punkt die Theorie von Dendy und Nicholls falsch ist") in attributing any sensory function to the sub-commissural organ. He believes that the sensory cells connected with Reissner's fiber are found only in the epithelium which lines the central canal and holds, with Sargent, that the sub-commissural organ serves merely for the support or anchorage of the fiber. These sensory cells are

described by Tretjakoff as projecting into the lumen of the central canal where each is said to end in a small knobbed process, which Tretjakoff compares to the bellpush of an electric bell. He supposes that the stimulation of these cells is effected by the pressure of the fiber upon these processes whenever the body is flexed.

That Tretjakoff's investigations were made upon material in which the fiber had been broken and had retracted is suggested by his figures. Two only of these depict the central canal. In one (fig. 20) the fiber (which is invariably very fine in the Ammocoete) is seen indistinct and vastly swollen. In the other (fig. 19) the fiber is absent and the lumen of the central canal is occupied by nuclear bodies, the remains probably of epithelial cells dislodged from the ependymal epithelium by the fiber in its withdrawal. Under these circumstances it is not surprising that Tretjakoff failed to find the delicate filaments which seem to join the fiber at frequent intervals as I have described ('12 a) and the occurrence of which has been confirmed by Studinčka ('13, p. 585).

The little knobs (Tretjakoff's bell-pushes) are almost certainly the retracted remnants of the fibrillae of those cells which, in my view contribute to the formation of the fiber and which, torn free by the dislocation of the fiber, have shrunk back upon the sensory process of the parent cell.

B. Earlier attempts to determine the function of Reissner's fiber by experimental methods

The first reference to experiment in connection with the question of the function of Reissner's fiber occurs in a preliminary paper by Sargent ('01). These experiments were subsequently described in greater detail in 1904.

In these experiments an attempt was made to break the fiber by a means of incision made through the choroid plexus of the fourth ventricle of certain elasmobranchs. Such experiments, involving, as they necessarily did, the risk of serious disturbance to the central nervous system or even actual injury to the brain

itself, were of little value, for it could not be established that any of the reactions observed were the results simply of the interruption of the 'optic reflex short-circuit' alleged to be provided by Reissner's fiber.

I gather, moreover, that Sargent relied upon observations made from dissections to determine whether or not the experimental incision had really broken the fiber, which appears to me as an altogether unsatisfactory method. Whether there was a subsequent microscopical examination of the material is not clear nor does Sargent state what precautions were taken to prevent a disturbance of the fiber during the dissection. The statement that "the cord and medulla of each individual was preserved for microscopical examination" suggests that a part only of the nervous system was subsequently cut out. If this were the case, it is practically certain that, whatever the result of the experiment upon the fiber, it would be found retracted in the preserved material.

It is, therefore, a little difficult to ascertain the grounds for his remark ('01, p. 450) that "animals on which the equivalent operation was performed without breaking the fiber are nearly or quite normal."

Other experiments were made by Sargent ('01) to determine the effect of artificial extirpation of the eye upon the fiber but the results obtained were never recorded. Several years later, experiments were made upon Reissner's fiber by Horsley ('08). In this case the subjects of the experiments were individuals of two species of Macacus. Minute electrolytic lesions were made in the spinal cord, at the level of the fifth cervical segment, in order to break the fiber. No observations are recorded, however, upon the behavior of the living animals nor are details given as to the duration of the experiments. Concerning the appearance of the fiber under the microscope, Horsley remarked that Wallerian degeneration was not observed in the broken fiber.

I find, however, some little difficulty in interpreting the appearance of Reissner's fiber in the sections figured by Horsley.

In his figure 10, Reissner's fiber is seen in transverse section, occupying quite an appreciable part of the lumen of the central canal. As it is traced backwards from this level (the first cervical segment) through the third cervical segment (fig. 9) towards the point of lesion in the fifth cervical segment (fig. 8), it is seen to constantly diminish in size. Behind the point of lesion this diminution in size continues as will be seen in figures 11 and 12 but, more caudally, the diameter of the fiber is again seen to increase (fig. 13), this latter figure representing a section through the spinal cord in the lumbar region.

Now this is not at all what one would expect to find where Reissner's fiber had been broken experimentally. Usually it would be found that on either side of the lesion there was a stretch of canal devoid of fiber. Still further from the lesion the severed ends of the fiber might be found swollen and perhaps knotted if the material were killed and fixed soon after the lesion had been made. Tracing the fiber distally, in either direction, from these knotted or swollen ends one would expect to find that the fiber diminishes in diameter until the normal size is reached. If, however, the killing of the material were postponed for a considerable time after the experimental operation the swollen end and the spiral twisting would have disappeared and the fiber would have straightened out backwards, extending practically to the point of lesion, nearly normal except that it might not have regained its taut condition. The piece lying posterior to the lesion might have retracted wholly backwards to the end of the cord. If the material were not killed until several weeks after the operation it is probable that regeneration would have largely re-established the normal condition throughout.

The condition figured by Horsley, in which the fiber is most swollen anteriorly, regularly diminishes in diameter towards and past the lesion (and probably becomes normal in the thoracic region) but shows a renewed swelling very far back, suggests that the condition of the fiber may have had nothing to do with the actual experiment. I should judge that sufficient time had elapsed after the experiment to permit of regeneration, and the

reception of the fiber. Emerging from the anterior end of this groove, sometimes as a paired structure ('12 a, figs. 10, 11), Reissner's fiber stretches freely through the midbrain ventricle to the neighborhood of the posterior commissure.

The ventricular surface of the posterior commissure is clothed by a band of highly developed epithelium which is often folded in both the longitudinal and the transverse planes. It is to this remarkable tract of epithelium that the name 'sub-commissural organ' has been given. Owing to its longitudinal folding it has usually, in transverse sections, a horseshoe shape and partly encloses a median dorsal groove (the 'sub-commissural canal'). Reissner's fiber, if it has continued as an unpaired structure so far forward, breaks up at the hinder end of the posterior commissure into two or more strands which subdivide within this median groove into numerous delicate fibrillae which are connected with the cells of the sub-commissural organ.

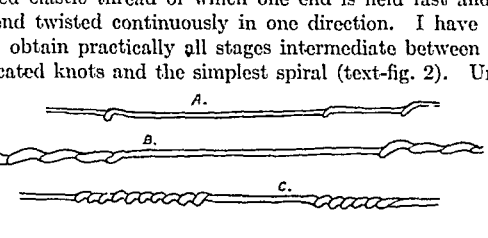
A study of the development of the fiber indicates that it arises by the confluence of numerous filaments springing from sub-commissural organ and that the composite thread so formed extends backwards into the central canal of the spinal cord. Within the central canal it probably receives numerous additional components from scattered cells in the epithelium which lines the central canal.

Perhaps the most remarkable characteristic of the fiber is its extreme elasticity. In life it appears to exist under quite considerable tension and to be somewhat prone to accidental breakage. In that event, or following artificial section, the free ends may recoil sharply to form tangled knots or 'snarls.' The retraction is accompanied by a marked increase in the diameter of the fiber.

This elasticity usually disappears very rapidly during the process of fixation and the preserved fiber may become distinctly brittle (fig. 21). If, however, the fiber be severed before fixation is completed a retraction will still take place, but much more gradually, and it will then be found that the fiber has become wound in a more or less open spiral. Even where the recoil has been an abrupt one, resulting in the formation of the

characteristic knot, a careful examination of this mass will, almost invariably, reveal the fact that the retraction was accomplished by a spiral winding of the fiber.

Such a knot of retracted fiber has, indeed, the form of a contorted mass similar to that which may be produced in any thin stretched elastic thread of which one end is held fast and the other end twisted continuously in one direction. I have been able to obtain practically all stages intermediate between such complicated knots and the simplest spiral (text-fig. 2). Unlike



Text-fig. 2 Stages in the twisting of Reissner's fiber in its withdrawal from the point of breakage. *A*, *B*, *D* from Scyllium canicula (9); *C*, from Petromyzon fluviatilis; *E*, *F*, *G*, *H*, from Raia blanda (3).

the simple twisted elastic thread, however, the spiral winding may appear interruptedly in Reissner's fiber, spiral stretches alternating with swollen but untwisted lengths. Moreover, the twisting does not always make its appearance at the free end but may arise at a greater or less distance from the point where the fiber has been broken.

If, therefore, the spinal cord has been cut prior to fixation, Reissner's fiber may be found to have withdrawn for a relatively considerable distance from the point of section and a great stretch of the central canal may be found devoid of fiber. The extent of such retraction apparently varies with the region in which the

fiber has been broken and depends, possibly, upon the size of the central canal in that particular region for, in the case of a sudden recoil, the spiral winding may produce at or near the severed end a mass of coiled fiber which apparently checks further retraction. With the retreating end of the fiber may be dragged numerous epithelial cells and, around it, will collect a quantity of coagulum (fig. 17) which may render it difficult to distinguish exactly the condition of the knotted end.

On the side of the tangle remote from the point of section, the fiber usually emerges as a coiled thread and thence passes gradually into a more open spiral. If the fiber has been cut at a sufficient distance from its attachment, this open spiral may pass into a swollen but straight stretch and ultimately be found to pass almost or quite into the normal condition. Broken, however, near to one of its attachments, the fiber will almost certainly withdraw violently and completely to that attachment, from which it may even tear itself free, dragging with it many of the epithelial cells.

While this retraction which is so characteristic of Reissner's fiber is, as I have pointed out ('09), altogether unlike anything known in a nerve, neither does it altogether resemble the recoil of a simple (homogeneous) elastic thread. It is, therefore, of especial interest that I have been able, recently, to detect in a greatly swollen and retracted fiber what appears to be a fine deeply staining central axis (fig. 17); the resemblance of the fiber to the stalk of a Vorticella (with which I have already compared it, '12 a, p. 25) is thereby greatly enhanced. This appearance is somewhat inconstant and never to be made out in the unrelaxed condition.

I have been unable to decide whether the numerous delicate fibrillae (fig. 29) seen in the central canal of the spinal cord are in organic continuity with Reissner's fiber or whether they are merely unusually long cilia which have been cemented to the fiber after death by the coagulated cerebro-spinal fluid. That the former view is probably correct is indicated, I believe, by the fact that in the cases in which some retraction of the fiber has occurred it is very rare to find any of these fibrillae apparently

related to the swollen and retracted portion of the fiber. Instead, in the region in which there has been a dislocation of the fiber, minute spherules of some highly refracting substance are found plentifully, close to or in contact with the free surface of the ependymal cells. That these are the contracted remains of such connecting fibrillae, which were, indeed, component filaments of Reissner's fiber is therefore extremely probable. The withdrawal of the fiber would inevitably snap such connecting filaments in the region affected and these broken protoplasmic strands would naturally shrink backwards towards the surface of the parent cells.

The view that Reissner's fiber is a thread of modified protoplasm, formed by the complete coalescence of numerous delicate filaments (or hypertrophied cilia) is indicated by its origin and is confirmed by its staining reactions. Moreover, it is an interpretation which renders comprehensible its singular elastic recoil notwithstanding its apparent structureless condition. Such spiral retraction is met with only, so far as I am aware, in the little differentiated protoplasm of the Protozoa, among which group the fusion of cilia is also no uncommon feature.

II. THE SCOPE OF THE PRESENT INVESTIGATION

From what has been stated above it will be seen that, while there has been a great variety in the suggestions made as to the nature of Reissner's fiber, there have been put forward but three theories as to its function.

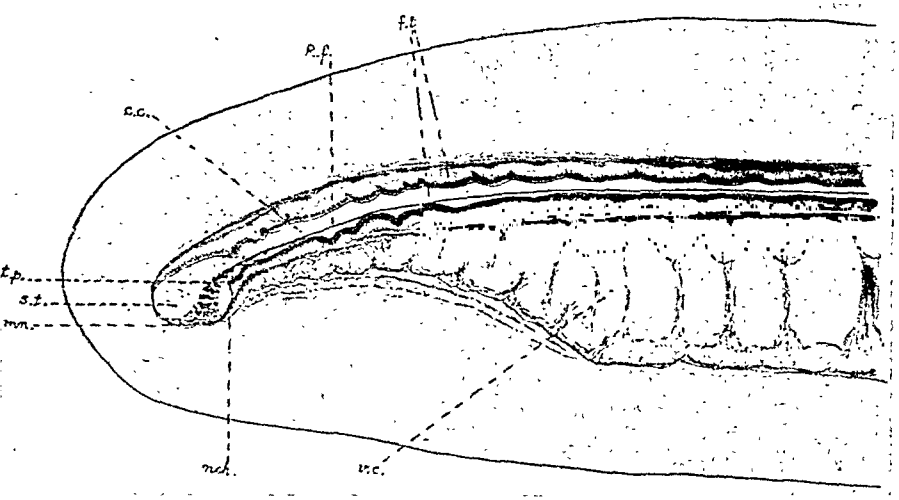
The disproof of Sargent's statements as to the nature of the fiber disposed, at the same time, of his 'optic reflex theory.'

Ayer's suggestion was based, as I have shown, almost entirely upon an erroneous idea of the nature and normal condition of the fiber and its relation to the ventricles; in any case his view is not one which could easily be tested experimentally.

There remained Dendy's theory which might readily be put to the test of experiment if a way could be devised of breaking the fiber without damage to the central nervous system.

Such an operation became possible with my discovery ('10, p. 527) of the actual condition of the hinder end of the filum

terminale in the Ichthyopsida. Elsewhere completely enveloped by the brain and spinal cord, Reissner's fiber is peculiarly accessible at the extremity of the tail, the more so that there is practically an absence of nervous tissue in the hinder part of the filum terminale. This structure is, indeed, little more than a simple tube of columnar epithelium. At the actual hinder end, Reissner's fiber may be said to be protected only by the



Text-fig. 3 A sagittal section through the extremity of the tail of Raia blanda (III—experiment 3) to show the position of the sinus terminalis. *c.c.*, central canal of the spinal cord (and terminal filament); *f.t.*, filum terminale; *mn.*, meninges, forming the hinder wall of the sinus terminalis; *nch.*, notochord; *R.f.*, Reissner's fiber; *s.t.*, sinus terminalis; *t.p.*, terminal plug; *v.c.*, vertebral column.

skin and the delicate meninges, between which there lies but a film of connective tissue (text-fig. 3).

A cut made in the vicinity of the end of the terminal filament would break the continuity of the fiber, therefore, but would be quite unlikely to produce physiological results such as to mask or interfere with the reactions resulting from the disorganization of the mechanism of which Reissner's fiber forms a part.

My experiments, then, were intended primarily as an attempt to determine the function of Reissner's fiber and its related

structures by means of observations made upon the living animals in which the continuity of the fiber had been intentionally destroyed, but I had other objects, also, in view.

At the time when the experiments were undertaken practically nothing was known concerning the mode of recoil of the fiber. Sargent had stated that when cut before fixation the free ends of the fiber retracted into a knotted mass or 'snarl' but he had not observed that this snarl was spirally wound. I had myself seen such snarls in several cases in material which had not been specially preserved for the study of this structure and in which the spinal cord had been cut previous to fixation ('12 a, figs. 17, 18, 19). In most of such material the fiber was ill preserved and, in the main, my own attention had been confined to a determination of the normal anatomical relations of the fiber. Accordingly, I had taken special precautions to thoroughly fix and harden my material before severing the spinal cord. Nevertheless, I had come, in the previous year, upon a few examples of this spirally wound condition which had been obtained unintentionally by a premature cutting of the spinal cord ('12 a, figs. 12, 16). These accidents, however, had yielded no information concerning the behavior of the fiber cut in life. It was naturally supposed that a breaking of the fiber in the living animal would be followed by a sharp recoil of the severed ends similar to that which was known to occur when the fiber was cut in freshly killed material. It was desirable, however, to ascertain if this were so.

It was anticipated, moreover, that the results of the experiments would throw light upon the question of the natural limits of this recoil. It must be remembered that beyond the mere fact of the occurrence of a recoil nothing had been recorded, and it was not even known whether the recoil started by the section of the living fiber would continue until both free ends had retracted to their respective points of attachment or whether, on the contrary, there would be formed speedily, in the living animal, a tangle (or tangles) which might (on reaching a size sufficient to block the lumen of the canalis centralis) automatically check further recoil in one or both directions.

In the latter event the tangled end (or ends) might perhaps afford a temporary hold and so prevent the fiber from being put completely out of action.

And, finally, there was the problem of regeneration. It was uncertain whether a tangle, if it were formed, would remain as a permanent record of the breaking of the fiber, or, if it were a transient feature, whether it would simply uncoil or whether the whole fiber, or the tangled part of it, simply disappeared to be replaced by a new growth.

Upon some of these points a certain amount of light was shed by the results of the few preliminary experiments carried out in 1910 but upon others the information was too meager to supply a decisive answer. Upon many of these points much additional knowledge has been gained from the more extended investigation carried out in the following year.

III. MATERIAL AND METHODS

The curiously exposed condition of the filum terminale in fishes, coupled with the fact that in both elasmobranchs and teleosts, Reissner's fiber is particularly well developed, largely influenced my choice of material. My final preference for elasmobranchs was determined by the idea that the absence of bony tissue in the vertebral column would facilitate the preparation of the inevitable large number of series of sections.

I planned, originally, to experiment principally upon the common dogfish (Scyllium canicula) and to use rays only in the event of dogfish of suitable size being unobtainable. Knowing nothing certainly as to the probable extent of the recoil of the fiber, I was anxious to make use of comparatively small specimens, for it was possible that serial sections of the entire length of the central nervous system of all of the specimens might have to be prepared—a task of no little magnitude.

As it happened, only a couple of reasonably small dogfish were obtained during my stay in Plymouth in July, 1910; relatively small rays were, however, moderately plentiful, and for the most part the preliminary experiments were performed upon these animals.

Subsequent examination of this material under the microscope indicated that in most cases it would be necessary to examine only an inch or so of the spinal cord in front of the place where the incision was made. This point was almost always within a third of an inch of the extremity of the tail. The size of the specimen thus appeared to be of no great importance but this fact was only ascertained when the material had been prepared for microscopic examination nearly a year subsequently to the completion of these preliminary experiments.

Accordingly in the summer (August) of 1911 I was less careful to restrict my experiments to specimens of small size. I was thus enabled to obtain, more readily, the many specimens which I required. In all, a dozen comparatively small dogfish, ranging from 14 to 20 inches in length, were secured, and, upon these were performed experiments varying in duration from a few (three) hours in some cases to more than eighteen days in others. Of the rays, three species were employed, but of one of these, Raia microcellata, I had but a single specimen and, as in the previous year, the greater number of the experiments were made upon specimens of R. clavata and R. blanda. These included rays which were barely 6 inches in length and which were, presumably, just escaped from the egg case, while others ranged up to 16 inches. The duration of the experiments, in the case of the rays varied from a few (ten) minutes to as much as thirteen days.

The actual operation consisted in severing Reissner's fiber at a point quite near to the hinder end of the terminal filament and was practically nothing but a simple prick which rarely drew a drop of blood although, in some cases, the sections showed that there had been some effusion of blood into the canalis centralis.

Notwithstanding its trivial character, however, I was obliged by the conditions under which the vivisection license was issued, to perform the operation only upon anaesthetized specimens.

Some trial experiments with the anaesthetic indicated that dogfish were curiously susceptible to chloroform and, despite

my precautions, two of the subjects of the experiments subsequently failed to recover from the anaesthetic.

Finally it was found that a short immersion of the specimen in sea-water in which had been shaken up a small quantity of a mixture of chloroform and ether would induce a sufficient degree of insensibility, and this method was adopted throughout my series of experiments in 1911. Under this treatment, none of the specimens died.

The operation was quite easily performed, the subject being removed from the chloroform water and placed upon the table with its tail turned upon the side. The necessary prick was inflicted with the point of a very fine scalpel (which had previously been sterilized by passing through a gas flame) at a point usually considerably less than a third of an inch in front of the sinus terminalis. In the dogfish, therefore, the incision perforated the caudal fin near its hinder border while in the rays the cut was generally made behind the last dorsal fin (figs. 2, 8). The animal was at once returned to its tank, having been out of water for, perhaps, thirty seconds. Recovery was usually rapid and, as might be expected, there was no evidence of shock.

None of the specimens died from the effect of the operation, nor in the subsequent examination of the tissues in serial sections, was there found any indication that morbid or septic conditions had been set up. Indeed, apart from certain peculiarities of behavior about to be described, and which I attribute to the breaking of Reissner's fiber, the animals suffered no apparent ill-effects.

Nevertheless, two or three specimens were lost during the progress of the experiments from causes indirectly connected with the experiments. In the second series of experiments a number of photographs were taken, of normal specimens as well as of the subjects of the experiments. I could find no record of previous attempts to photograph living fish, and had accordingly to make a number of trial exposures. At first, attempts were made to obtain the photographs out of doors by daylight. Numerous difficulties cropped up however, for none of the out-

side tanks were glass fronted, and the only available glass-fronted tank, of a size to be readily transported, held but a comparatively small quantity of water and there were no facilities for connecting this tank with the aerating apparatus. A prolonged sojourn of the fish in this tank was not possible so that attempts to photograph under these conditions involved disturbing the specimens, transferring them in a bucket to the small tank and then waiting for them not only to settle down but to settle in a position in which it would be possible to photograph them. One or two lucky snapshots were obtained but the method was, in general, a failure.

An attempt to photograph the fish in their proper tanks in the laboratory encountered other difficulties. Of these the chief was connected with the light. With subdued daylight a comparatively long exposure was needed and it was found in practice that the head region was always blurred by the respiratory movements even if the fish did not elect to move bodily during the process.

In the end flash-light photographs were taken. The camera was fixed up opposite the tank in which was the specimen of which a photograph was desired and by the light of an incandescent gas lamp it was focussed upon a part of the tank a little within the glass front. Above the camera was stretched a piece of string upon which were placed a number of bent strips of magnesium ribbon. Usually some twenty inches of the ribbon were required, divided into four or more pieces. The gas lamp was then extinguished, and the aerating tube and bulb removed from the tank to do away with movement in the water. As soon as the specimen settled in a suitable position the strips of magnesium were lit, as nearly as possible, simultaneously. The reflection from the glass front of the tank was considerable, but in some of the later photographs this was diminished by igniting other strips of magnesium suspended immediately above the tank, care being taken to shield the lens from the direct rays from this source of illumination. Most of the photographs reproduced here were taken in this way.

In all, experiments were performed upon sixty-seven elasmobranchs, of which twelve were dogfish and the remaining fifty-five were rays. Two only, as already mentioned, died from the effect of the anaesthetic, while two others died from suffocation consequent upon my omission to replace the aerating tube in the tank after the specimens had been photographed.

They were killed by being plunged into a mixture of spirit and chloroform and, after a brief stay in this fluid, were eviscerated. In this way the blood vessels were practically drained, which greatly facilitated the rapid dissection necessary to expose brain and spinal cord, there being no troublesome effusion of blood from cut vessels within the brain case. The partially dissected specimens were immersed in a large vessel of fixing fluid (Tellyesnicky's bichromate-acetic mixture) and the further dissection required to expose the greater part of the spinal cord was completed under the fluid. To dissect away the vertebral column from the hinder part of the spinal cord and the filum terminale is, however, a very delicate operation, which involves considerable risk of damaging the nervous system. The exposure of the spinal cord was, therefore, carried only to within a couple of inches of the end of the tail. Behind this point I was content to strip away most of the skin and muscles, about half an inch at the actual extremity being left quite untouched. In the case of the dogfish the last inch (or even more) of the tail was left intact.

The preparation of the series of sections proved unexpectedly difficult. In general, a piece of the tail, about an inch in length, was removed—this piece including the point of experimental lesion—and prepared for sectioning.

My intention was to cut this terminal piece sagittally in order that the point of experimental incision and a considerable length of the filum terminale before and behind this point might be seen in one and the same section. To avoid risk of damage to the sinus terminalis it was found expedient to retain undisturbed the skin upon the last half inch or so of the tail and the terminal piece, therefore, contained the bases of numerous spines embedded in the skin, and separated from the axis of

partly calcified cartilage by particularly tough connective tissue with contained fin-rays. These several structures became greatly indurated during the prolonged paraffin embedding which was found to be necessary. Moreover, the various tissues contracted unequally during this process with the result that despite many precautions a very troublesome crumpling was often produced.

This was most in evidence near the actual extremity of the tail and thus affected, principally, the region behind the incision so that, while it was usually easy to determine if the fiber had retracted backwards from the lesion it was sometimes extremely difficult to certainly recognize the contracted piece of fiber. Especially was this the case when a considerable infiltration of blood into the sinus terminalis had accompanied or followed the recoil of the fiber.

In such sections, the filum terminale appears as a number of isolated pieces, often cut quite obliquely and a diagrammatic sagittal section through the sinus terminalis, such as that seen in text-figure 3, was but rarely obtained.

Apart from this crumpling the tail usually becomes bent at the place where the incision was made, so that the lengths of filum terminale before and behind the incision rarely lay in the same plane, notwithstanding that weights were used during the process of embedding to keep the tissue as nearly flat as might be. In front of the experimental incision the crumpling was less noticeable, the vertebral axis being more rigid, and the muscular and other soft tissues liable to contraction having been, for the greater part, removed. Nevertheless, even here, a certain curvature almost invariably occurred. Further, the greater hardness of the cartilage in this region often caused the sections to cut very unevenly. This irregularity could be largely avoided, it was found, by cutting rather thick sections (not less than 30 μ). The lumen of the central canal, however, in the hinder part of the spinal cord of the rays examined has a diameter which rarely exceeds 30 μ and in such sections, therefore, the whole of the central canal may be included within the thickness of a single section or a relatively thick layer of overlying tissue may seri-

One other point must be mentioned here. In the ray the actual position of the terminal sinus varies slightly, it was found, in different individuals. In the case of the specimen of Raia blanda figured (text-fig. 3) this terminal chamber extended downwards *behind* the extremity of the notochord, which is, I believe, the strictly primitive condition. It occurs, however, less frequently in this position than might be expected, and in many cases it lies altogether dorsal to the notochord, not always extending even to the posterior extremity of that structure. Whether there has been some mutilation in these cases or whether on the contrary there takes place, normally, a certain amount of resorption of the tissue of the terminal filament, I can not decide.

In some teleosts I have found what are, almost certainly, stages in the disappearance of the postero-ventral (post-chordal) part of the neural tube. I find, moreover, that the corrugation of the hinder end of the filum terminale in small rays which I have described ('12, p. 423) as so strongly suggestive of neuromeric constriction, is likewise frequently met with in the vanishing vestiges of the filum terminale in the region of the disappearing tail in the recently metamorphosed anuran.

While these facts suggest that the variation in position of the sinus terminalis of the ray may be due to some extent to the absorption of tissue in this region,[1] the possibility of mutilation must not be ignored. The actual end of the tail of the ray is soft and not protected by spines, and specimens which have suffered quite considerable mutilation are by no means rare. The terminal sinus, too, in those specimens in which it lies wholly dorsal to the notochord (fig. 19) rarely shows that bulbous expansion which is seen in examples in which the sinus terminalis has the postero-ventral position (fig. 20) but has quite a marked resemblance, in shape, to the secondary terminal sinus which I

[1] That an absorption of tissue in this region does occur in rays is suggested by Beard's statements ('96, p. 55, footnote 2), that the young (Raia radiata) immediately prior to escape from the egg case are shorter by a centimeter or so than embryos a month younger. Some of my own specimens which were six inches or less in length must, almost certainly, have been quite newly escaped and the process of resorption was possibly incompleted.

have found produced as the result of my experiments ('12, text-fig).

Be the reason for this variation in position what it may, it has a certain importance in this investigation, for in one or two cases where the sinus terminalis lay unexpectedly far forward, the incision (which was made in the postero-ventral region of the tail, being planned to break the fiber actually in the sinus terminalis) missed the terminal filament altogether.

Of young dogfish, only recently emerged from the egg-case, I have had no material but in the adult there appears to be little variation in the position of the terminal sinus.

In several cases, both dogfish and rays, the cut was made in the region of the terminal filament but just a trifle too far dorsally, and the sections show that, although the cut penetrated the neural canal, the filum terminale and surrounding pia mater escaped damage.

Such specimens in which the experimental incision failed to break the fiber served well as control specimens. Other control specimens were simply anaesthetized without undergoing the usual operation. These latter on recovery behaved in perfectly normal manner.

IV. OBSERVATIONS UPON THE LIVING ANIMAL

1. Upon normal material

The experiment carried out in 1910 had almost immediately directed my attention to the fact that a frequent, if not an invariable, consequence of the operation was the assumption by the subject of the experiment of a very distinct attitude while at rest. Accordingly, during the time spent at Plymouth both in 1910 and 1911, while the experiments were going on in the laboratory, very constant and careful attention was given to the numerous normal specimens which were kept in confinement in the adjoining aquarium. Control specimens, too, were kept under observation in small tanks in the laboratory under conditions precisely similar to those in which the subjects of the experiments were maintained.

quently followed by a period of marked activity. In this case the animal would dash about the tank, commonly blundering heavily into the confining walls. This phase rarely endured for long, but gave place to a quiescent stage in which the animal apparently exhibited a preference for the darker part of its tank. Settling down, it might remain inactive for comparatively long periods, moving only when disturbed. In other cases the specimen, recovering from the anaesthetic, passed directly into this lethargic condition. I imagine that this difference in behavior was due to the varying degree in which the animal had been affected by the anaesthetic, a slight degree of insensibility being marked by the erratic activity when volition was recovered.

Be this as it may, the assumption of some posture of the body unlike that which I have described above as normal, was frequently manifested very soon after the quiescent stage was reached. In some cases it appeared within ten minutes of the operation. Both the head and tail would be gradually lifted until the long axis of the body, from being a straight line would become markedly curved (figs. 2–5). The tail was, in general, sharply upturned from its base, while the trunk region was uplifted upon the pectoral fins from a region just behind the head. In the rays, owing to the great development of the pectorals, this appears to give rise to a transverse curvature of the anterior part of the body, as seen from in front (figs. 9, 13, 14).

There may be also a distortion of the long axis in the horizontal plane, the trunk and tail being bent several times from side to side (in some of the dogfishes) or with a single sharp bend of the hinder part to one side (rays and dogfish).

It is probable that a disturbance of the poise of the body exists, likewise, while the animal is in motion. It is, however, very difficult to be sure of this. In some of the dogfish, certainly, uniform undulation of the body in swimming seemed to be replaced by a less even movement which is perhaps best described as a wriggling action.

These reactions did not always make an appearance quickly after the operation. In some cases their advent was delayed for days even, and in yet others, as will be seen from the detailed

record given below, they never appeared at all. The explanation of these apparent exceptions must be deferred until after the account of the microscopical examination of the experimental material.

The duration of the reaction also varied considerably, persisting in some cases for a few hours only, while in others it endured for several days. In a few cases it appeared to be intermittent.

V. A SUMMARY OF THE RECORD OF THE EXPERIMENTS AND AN ACCOUNT OF THE EFFECTS UPON REISSNER'S FIBER

A. Scyllium canicula

2. The experimental incision was made at noon on July 7, 1910. The specimen quickly recovered and, although somewhat sluggish, appeared to swim normally. At rest, its body was bent slightly but otherwise the posture seemed normal. No change was observed until July 11, when the ventral border of the caudal fin was seen to be lifted slightly (about half an inch) from the tank floor. The animal became more sluggish and, if disturbed, soon returned to rest, exhibiting an apparent preference for the darkest corner of its tank, which rendered observation more difficult. The tail rested, moreover, against a sloping part of the tank where wall and floor met. It was impossible, therefore, to be sure whether the tail was really slightly lifted by muscular effort or merely upraised on account of the elevation of its support. The whole body, however, was seen to be considerably curved. On July 13 and 14 the fish was more restless and upon the 15th, when seen at rest, the long axis of the body was disposed in a straight line (for the first time since July 8). During the following day it was noticed that the body of the fish was once more bent from side to side in long wavy curves, but with the tail, as before, supported upon the sloping part of the tank. Next day, however, it was found resting well away from the back of the tank and the tail was uplifted, a clear two inches, from the floor. By midday on the 18th this reaction was still more marked and the hinder part of the trunk and tail were bent sharply to one side. Throughout the two succeeding days this reaction was pronounced. On July 21 the fish had reverted to an earlier posture, with the tail supported against the sloping part of the floor, but by midday it was once again well out in the tank with the tail held well off the floor. On the next day the reaction was less marked though the body was still bent. During July 23 the reaction was scarcely discernible and later in the day, when it was decided to kill the specimen, the fish appeared normal. It showed very marked activity in its attempts to avoid the net, swimming with a wriggling movement (the head and forepart of the body being twisted quickly from side to side). This action in swimming had been

noticed on several previous occasions. This specimen, then, gave a marked reaction lasting for 13 days. Duration of the experiment 16 days +.

The sections showed that, in front of the incision, a secondary sinus terminalis had been produced into which Reissner's fiber is seen to extend and, in contact with the hinder (meningeal) wall of which, it flares. Apparently it has just become attached thereto (fig. 31). Behind the incision the fiber has apparently entirely disappeared.

9. This specimen, for nearly a week after the operation (performed on July 12), showed a curious restlessness, not once being observed at rest until the morning (10 a.m.) of July 18. This activity was followed by an equally marked lethargy. The specimen took up a position in the darkest corner of the tank, where it lay with the body bent upon itself at a sharp angle and the tail supported against the sloping part of the tank. Not until July 22 was the specimen seen, in repose, away from the wall of the tank when it was found resting with the end of the tail slightly lifted: the flexure of the body was nearly straightened out. It was killed on July 23 the experiment having lasted 11 days.

In the sections the fiber (in front of the lesion) is found to extend backwards nearly to the place where the filum terminale was severed. It is probable, therefore, that the fiber had nearly recovered from the effect of the operation but the experiment was ruined by an accidental cut made, far forward in the trunk region, when exposing the spinal cord. The fiber in the piece examined is much swollen and continuously twisted (text-fig. 2), undoubtedly due to a (backward) retraction from this distant cut.

20. The incision was made at 11.15 a.m. on August 3, 1911, and, by noon, the tail was lifted slightly so that the lower border of the caudal fin no longer rested upon the tank floor. When disturbed, the fish swam with a quick wriggling action (cf. 2) and came to rest in a curious attitude in a corner of the tank, the anterior part of the trunk being poised vertically, supported by the adjacent walls of the tank, while the posterior part lay out horizontally upon the tank floor (cf. the ray, fig. 11). After being again disturbed, it once more came to rest in this peculiar attitude and so remained until 3 p.m. at which time it was again compelled to move. It was observed to swim in quick rushes, even leaping partly out of the water, and the wriggling movement was very noticeable. Ten minutes later it had settled down with the end of the tail slightly lifted but resting lightly against the tank wall. It was disturbed yet again and was subsequently induced to settle well away from the walls of the tank and the tail was then seen to be held at least an inch and a half from the floor, and it continued in this attitude until 4.15 p.m. when it was accidentally disturbed. During the next half hour it was repeatedly set in motion by the movements of another dogfish which shared the tank. It settled down six several times in the same attitude (with head and tail lifted) once or twice essaying the half vertical position which it had

assumed earlier. By 4.45 p.m. the tail was lifted more than two inches from the floor. The specimen was then driven about the tank and compelled to swim actively for several minutes and then removed and killed. There was in this case a well marked reaction which endured for the entire period of the experiment—5½ hours.

In front of the lesion the fiber has completely withdrawn from the piece of terminal filament and spinal cord examined.

21. The incision was made at 11.40 a.m. August 3. Upon recovery from the anaesthetic the specimen adopted the normal attitude. It was killed at 10.30 a.m. August 6, having given no apparent reaction during the three days of the experiment.

The sections are poor but show that the incision missed the terminal filament and thus failed to break the continuity of the fiber which is seen to be of normal diameter and to lie tautly stretched.

22. Within 10 minutes of the operation (performed at 10 a.m., August 4) a marked reaction appeared, the lower border of the caudal fin being lifted a clear two inches from the tank floor. The animal was sluggish and, after being disturbed, reverted always to this attitude. It was twice photographed later in the afternoon but the reaction had then become less marked (figs. 2, 3) but continued as shown until the specimen was killed at 5 p.m. Duration of the experiment 7 hours.

The fiber has apparently been withdrawn forward from the lesion completely beyond the anterior limit of the piece of spinal cord sectioned.

23. The incision was made at 10.10 a.m. August 4, but was followed by no apparent reaction and the specimen continued normal until it was killed on August 22. It was photographed on August 7 (fig. 6). Duration of the experiment 18 days 8 hours.

The sections show that the cut failed to penetrate the neural canal and the normal Reissner's fiber may be seen lying tautly stretched in the central canal of the undamaged terminal filament.

24. The incision was made at 4 p.m., August 4, and the usual reaction was noticed within half an hour of the operation, the caudal fin being lifted two inches or more. It was, however, less sluggish than the subject of the preceding experiment and frustrated all attempts to obtain a photograph during the early days of the experiment. The reaction continued uninterruptedly until the evening of August 8, the photograph (fig. 5) being obtained about midday on August 6. From the 9th onwards the reaction appeared intermittently and during the whole of the 10th the caudal fin was observed to be resting lightly upon the floor of the tank though the head was still somewhat raised. Late in the evening of the 15th and again at noon on the 18th the tail appeared slightly lifted for a while, but for the most part the reaction rarely appeared for any length of time after the morning of the 14th August (the eleventh day of the experiment). The specimen was notably sluggish during the later stage of the experiment and passed most of the time in a corner of the tank, with the tail supported upon

the sloping surface there. It was killed at 6.30 p.m., August 22. Duration of experiment 18 days $2\frac{1}{2}$ hours.

The severed (hinder) portion of the terminal filament had not markedly disintegrated but the short length of Reissner's fiber separated by the incision has altogether disappeared. There is visible some disorganization of the terminal filament in front of the lesion, but a little in front of the point where the cut was made the lumen of the central canal seems to have been widened somewhat, perhaps, to form a secondary sinus terminalis. Stretching backwards to this point, there is seen a flimsy wrinkled and fibrillate structure which is, I believe, the expanded hinder end of Reissner's fiber.

34. The incision, which was made at noon, August 14, was followed within a quarter of an hour, by a distinct reaction (fig. 4). This continued and was well marked at 3 p.m. when the specimen was killed. Duration of experiment 3 hours.

In front of the region where the experimental incision was made, the fiber is found retracted and swollen with some spiral twisting. Behind the point of injury the fiber is markedly swollen and appears fibrillar.

33. The incision was made at 12.05 p.m., August 14, and by 1 p.m. a slight reaction had appeared, but for the greater part of the day, the animal rested with the tail turned over upon its side. The whole body was strongly curved. During the following day this same attitude was largely maintained but at times the tail was seen to be lifted considerably. The specimen was killed at 8.45 p.m., August 15, the duration of the experiment thus being 1 day $8\frac{3}{4}$ hours.

Reissner's fiber is found swollen, retracted forward from the region of the experimental incision and, at the free end, is slightly spirally wound. Traced forwardly this spiral becomes a very open one and the fiber passes into a comparatively straight course. It probably represents a stage in unwinding.

43. The incision was made at 11.20 a.m., August 18, and was quickly followed by the usual reaction, the head being well raised. By noon the tail, also, was well lifted and the head still further raised. At 7.15 p.m. when the specimen was killed, the reaction appeared less pronounced. Duration of experiment 8 hours.

In front of the lesion, Reissner's fiber had disappeared entirely from the length of tail examined. Behind the incision also, the fiber has evidently contracted, the canal being devoid of fiber nor can the contracted piece be certainly recognized.

44. The incision was made at 11.45 a.m., August 18, but no reaction appeared either upon this or the following day. The specimen was killed at 8 p.m., August 19. Duration of experiment 1 day $8\frac{1}{4}$ hours.

The fiber though severed has apparently been gripped by the adpressi/n of the walls of the terminal filament and there has been no retra%tion of the fiber in either direction (fig. 30).

B. Raia blanda

3. This specimen was one which failed to recover from the anaesthetic. Some 2 to 3 hours after the operation it appeared to be dead, the central nervous system, therefore, was partially exposed and preserved.

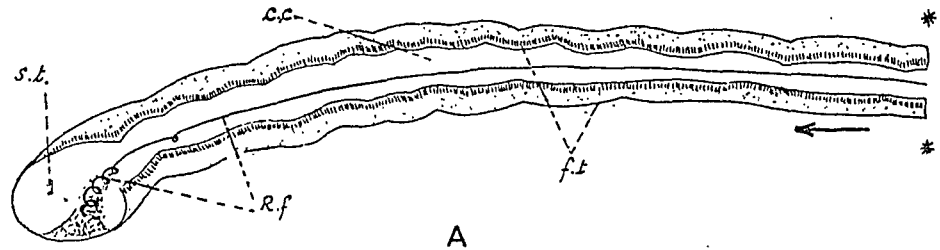
The sections show that the incision severed the fiber but at the same time apparently pinched together the walls of the terminal filament sufficiently to hold the cut ends. Behind the incision, therefore, the fiber is found, stretching backward from the region of the lesion to the sinus terminalis. It is somewhat swollen, the swelling becoming more marked as the terminal sinus is neared and, actually within the terminal chamber, it becomes greatly swollen and coiled. The terminal plug is obscured by this retraction and cannot be certainly identified (text-fig. 4).

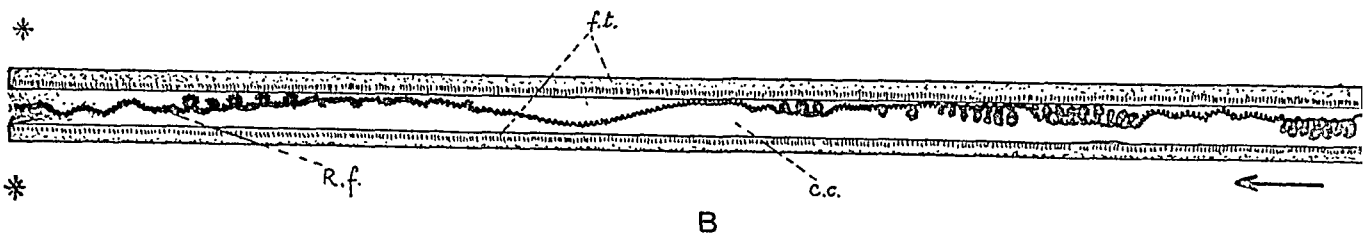
In front of the lesion Reissner's fiber is found, everywhere in the length of the terminal filament examined, much swollen and most remarkably coiled, all the later stages of spiral winding (short of the production of actual tangles) being found in this short extent of central canal (text-fig. 2). Regions in which the fiber is simply twisted alternate with others in which the coiling is quite complicated and it is probable that the original (uncontracted) length of the fiber included in the piece examined was many times that of the length (about $\frac{3}{4}$ inch) of the containing central canal. The evidence suggests, therefore, that there must have been in progress, at the time the incision was made, a very definite retraction of the fiber in a backward direction from a point well in advance of the experimental cut. This cut clearly checked further retraction behind the lesion, but in front the retraction *probably continued until it was stopped by the hardening* action of the fixing fluid several hours subsequent to the operation.

4. The incision was made at 10 a.m., July 8, and was followed by a quick recovery. Thereafter, the fish swam about with its tail turned dorsally. *Six hours later, when seen at rest, it was noted that its tail* was turned sharply to one side and that the extremity was raised at least an inch. The tail was still lifted at 9.30 a.m. next day but later this peculiarity was less pronounced. The specimen was killed at 3 p.m. Duration of experiment 1 day 5 hours. .

The sections are poor and very obliquely cut. A considerable clot occupies the central canal for some distance in either direction from the experimental lesion. *Behind the region of the incision* I have failed to recognize Reissner's fiber but in front it can be made out vaguely, apparently lying somewhat slackly against the epithelial lining of the central canal and with some trace of irregular swelling and coiling (fig. 16).

7. The incision was made at 10.30 a.m., July 9, and was quickly followed by an elevation of the end of the tail, the whole tail being turned slightly to one side (the left). *By July 11 the fish appeared to* have become normal, excepting that it continued to exhibit a prefer-





Text-fig. 4 A slightly diagrammatic median sagittal section through the filum terminale of Raia blanda (3) behind, 4A, and in front, 4B, of the experimental lesion. The arrows indicate the direction in which recoil of the fiber had taken place. *c.c.*, central canal of the spinal cord (and terminal filament); *f.t.*, filum terminale; *R.f.*, Reissner's fiber; *s.t.*, sinus terminalis; **, indicate the region of the incision.

ence for the dark corner of its tank and adopted the somewhat unusual action in swimming noted in no. 5 (Raia clavata). During the 11 succeeding days, it was observed at frequent intervals but was apparently normal throughout this period. On July 22 it was killed Duration of experiment 13 days +.

This tail was examined by means of sections cut transversely which proved to be quite unsuitable for the purpose of this investigation. The fiber is found in the more anterior sections, where it appears not markedly swollen and has apparently nearly made good any retraction which may have taken place after the operation.

8. The incision was made at 10 a.m., July 13. By 10.30 a.m. the specimen had completely recovered from the anaesthetic and had attached itself to the (vertical) glass plate forming the front of the tank, the tail being lifted slightly and turned to the left. This condition persisted for an hour or so but by midday the ray appeared normal. It was killed at 11 a.m., July 14. Duration of the experiment 25 hours.

There was no considerable retraction of the severed ends of the fiber, in either direction from the lesion, these being entangled apparently, in the clot which occupies the central canal for some distance. From this clot the fiber may be traced tautly stretched and of normal diameter.

10. The incision was made at 11.30 a.m., July 13, and was followed very quickly by a marked uplifting of the tail. An hour after the operation the ray was found adhering to the wall of the tank with the tail swung out dorsally and to the left. The reaction continued to be marked during the three following days. By the morning of July 17, however, the ray was seen with the tail carried normally and, thereafter, the specimen appeared normal until July 23 when it was killed. Duration of experiment 10 days.

For some distance in front of the experimental lesion, the central canal is found empty of fiber. The free end of the fiber is found, about half an inch in front of the lesion, swollen and thrown into a loose tangle (fig. 26), from the anterior end of which the fiber emerges much less swollen and fairly straight. No part of the fiber shows any trace of spiral twisting.

11. The incision was made at 10 a.m., July 20, with a fine knife from the right side, care being taken not to penetrate completely through the tail. The usual reaction did not appear, but there was some displacement of the right pectoral fin which was brought up sharply dorsally. Next morning the ray appeared entirely normal. It was killed at 4 p.m., July 22. Duration of experiment 2 days 6 hours.

The sections show that the incision missed the filum terminale and the fiber, therefore, remained unbroken.

19. The incision was made at 4.10 p.m., August 2. The specimen was kept under observation until it was killed on August 9 but during the whole of this time nothing unusual in its behavior was noted. Duration of experiment 6 days 20 hours.

The filum terminale behind the lesion appears empty of fiber but an indistinct mass, which is apparently a tangled heap of fiber is seen in the sinus terminalis. In front of the lesion the fiber is slightly withdrawn, the end being swollen and somewhat spirally coiled.

29. The incision was made at 4.20 p.m., August 7, and the ray was killed at 7.15 p.m. on August 10, no reaction having appeared in the meanwhile. Duration of experiment 3 days 3 hours.

The sections establish that the incision failed to sever the filum terminale and the fiber which is unbroken maintains its normal diameter and is seen tautly stretched.

41. The incision, made at 5.55 p.m., August 17, was followed, very quickly, by a reaction. The snout was lifted markedly and the whole body was arched up. The ray was disturbed several times but invariably returned to rest in the same attitude. By 8.30 p.m. the reaction had become less pronounced and by noon next day, when the specimen was killed, it was much less marked. Duration of experiment 18 hours.

In the terminal piece of the tail, Reissner's fiber is found slack, swollen and retracted for some distance from the region of the experimental lesion. Another piece of the spinal cord, taken some considerable distance in advance, showed the fiber very slightly slack and little swollen.

49. The incision was made at 11.15 a.m., August 21. A marked reaction very quickly appeared, affecting the pose, both in swimming and at rest. At noon, the snout and tail were down but the body remained curiously humped up. The specimen maintained this attitude until it was killed at 12.45 p.m. Duration of experiment $1\frac{1}{2}$ hours.

A conspicuous clot has formed in the region of the lesion and extends into the central canal both before and behind this point. Reissner's fiber is seen extending backwards from this spot as a swollen, loose and slightly knotted thread. In front of the incision, the fiber emerges from the clot (fig. 22) markedly swollen and coiled interruptedly, in which condition it continues throughout the entire length of the piece of spinal cord examined. The penultimate piece reveals the fiber still more swollen and more markedly twisted. It is clear, therefore, that although there has been no withdrawal, in either direction from the region of the experimental incision, the fiber was, nevertheless, undergoing a marked contraction. The only possible explanation was that the fiber had been broken farther forward and that a backward recoil had been set up, that having begun probably at or about the time of the operation. To test this point, another piece of spinal cord was taken from a place well forward in the trunk. The sections showed that, here, the central canal was perfectly devoid of fiber, a flimsy hollow cylinder of coagulum (?) occupying the center of the canal (fig. 18).

51. The incision which was made at 11.30 a.m., August 21, was not apparently productive of any reaction. The specimen was killed at 5 p.m. on the same day. Duration of experiment $5\frac{1}{2}$ hours.

The tail had clearly been truncated earlier in life but had completely healed and a secondary sinus terminalis had been formed. The experimental cut failed to break the fiber which is seen of normal size and tautly stretched.

52. The incision was made at noon and was followed by a scarcely perceptible reaction. The ray was killed at 3.30 p.m. Duration of experiment $3\frac{1}{2}$ hours.

The fiber was severed by the incision but the free ends, which are slightly knobbed and swollen are entangled in a clot and thus, presumably, retraction has been prevented.

53. The incision was made at 12.05 p.m. and was quickly followed by a fairly definite reaction which, however, was not evident at 2.30 p.m. when the specimen was killed. Duration of experiment $2\frac{1}{2}$ hours.

The fiber is seen cut and slightly slackened but the free ends have been withdrawn only for a short distance from the lesion.

62. The incision was made at 8.30 p.m., August 21, and was seen to be followed by a marked swimming reaction but the specimen was not seen in repose, after the operation. It was killed at 10.30 p.m. Duration of experiment 2 hours.

The sections are poor but serve to show that the filum terminale was cut. No fiber can be made out in such parts of the central canal as I have been able to examine. It is probable that the fiber has retracted forward, beyond the anterior limit of the piece of tissue sectioned.

63. The incision was made at 11.10 a.m., August 22, and was followed by a marked swimming reaction. At 11.40 a.m. it settled down but the tail was not displaced. It was killed immediately. Duration of experiment 30 minutes.

The fiber had been cut and had, apparently, retracted forwardly, completely from the filum terminale in the piece of tail examined.

66. The incision was made at 11.35 a.m. and was followed by a marked reaction. The ray was killed at 12.20 p.m. Duration of experiment 45 minutes.

The fiber was cut and had retracted some distance forward. In the sections it may be seen lying slackly in an undulating course but is not appreciably swollen.

C. Raia clavata

5. The incision was made at 10 a.m., July 8. By 5 p.m. the hinder part of the tail was seen to be lifted and this reaction was manifested throughout the evening and became still more marked next day. On July 11, the specimen appeared very lethargic and, on every occasion after being disturbed, returned to rest in the darkest part of its tank. In swimming, the specimen would remain poised nearly vertically, with a curious hovering movement, for 10 minutes or more at a time, its tail being turned sharply dorsally. At rest, so far as could be seen, the tail was disposed normally, but next day it was held uplifted for

D. Raia microcellata

61. The incision was made at 7.15 p.m., August 21. At 10.30 p.m the tail was distinctly raised and remained so until the specimen was killed at 11 p.m. Duration of experiment $3\frac{3}{4}$ hours.

Sections prepared through the tail were useless. The brain was sectioned, sagittally, and showed the fiber lying in normal position, of usual size and apparently tautly stretched, so that if retraction of the fiber took place in the tail region, it had not extended forward to the head.

In all, serial sections were prepared of sixty-two specimens.[2] Of these, the microscopical examination showed that in one case (16) the hinder part of the spinal cord was in an advanced stage of degeneration due to an accident which must have occurred at some time prior to the experiment. The sections through the region including the point of injury were, in five cases (30, 50, 61, 63, 69), absolutely worthless and two others (32, 48) were somewhat fragmentary and of value only in establishing that the experimental incision had severed the filum terminale (and therefore Reissner's fiber), while in another instance (70) the sections are, for the most part, very thick and Reissner's fiber can be but doubtfully distinguished. In this case the experimental incision did not penetrate the filum terminale.

Sufficiently satisfactory sections were obtained, therefore, in fifty-three examples. Of these Reissner's fiber shows a most remarkable coiling in two cases (3, 49) which must be attributed to the breaking of the fiber very shortly before the experiment. In the former of these, moreover, the specimen never recovered from the anaesthetic and afforded, therefore, no reaction. Two experiments (9, 39) were vitiated by an accidental cutting of the spinal cord very far forward, while the fixation was incomplete and in both of these cases, also, an interesting spirally wound condition of the fiber was produced. Apart from these four experiments, in which there was definite evidence of an interference with the condition of the fiber before or after the experi-

[2] Four specimens (1, 25, 27, 31) which died during the progress of the experiment had been so long dead, apparently, as to be worthless for the purpose of this investigation. A fifth specimen (33) was unaccountably mislaid.

ment, there are three cases (19, 46, 55) concerning which I am in some doubt as to the correct interpretation of the sections.

Excluding for the present these seven experiments which, for one reason or another, are inconclusive, I have, I believe, very definite evidence concerning the condition of Reissner's fiber in no fewer than forty-six specimens. The conclusions at which I have arrived are based solely upon the reactions in these specimens, about which there appears to be no question.

The subjects of these forty-six experiments may be classified, according to the effect of the experiment upon Reissner's fiber, in four groups.

1. Six specimens (nos. 11, 15, 21, 23, 29, 51) in which it was found that the experimental incision missed the filum terminale and thus failed to break the fiber.

2. Nine specimens (nos. 8, 18, 26, 28, 36, 38, 42, 44, 45) in which the fiber, although broken by the incision, failed to retract forward, or in either direction. The severed end (or ends) were held, apparently, by the adpressed walls of the filum terminale or, in some cases, secured from subsequent slipping by the clotting of blood which had escaped into the central canal from the cut meningeal vessels.

3. This, the largest group, includes thirty specimens in which a more or less extensive retraction of the fiber had followed upon the experimental incision. While in some individuals (37, 52, 53, 64, 66) this retraction was not very great, in others (10, 17 20, 22, 34, 35, 40, 41, 43, 54, 56, 57, 58, 59, 60, 62, 65, 67, 68) it was very considerable. In at least five (4, 5, 6, 7, 24) it may have been very extensive, also; but, if so, it had been largely repaired before the termination of the experiment.

4. A single specimen (2) in which the process of regeneration was apparently almost completed.

VI. THE RELATION BETWEEN THE CONDITION OF REISSNER'S FIBER AND THE REACTION OBSERVED

1. In the subjects of the experiments

1. Of the six specimens included in the first group two were dogfish. The duration of the experiment varied from a little less than 6 hours (51) to nearly 19 days (23). In not one of these specimens, in which the experimental incision failed to break the fiber was there any reaction.

2. In the second class come nine specimens in which, although the experimental incision was successful in breaking the fiber, this did not undergo retraction forward from the lesion. All but one of these specimens were rays and the duration of the experiment varied from three-quarters of an hour (38) to nearly 7 days (18). For the most part, however, the specimens were killed in the first or second day.

Six specimens were not visibly affected by the operation, while the remaining three exhibited a scarcely perceptible reaction. This took the form either of a very slight uplifting of the tail for a quite brief period (8) or of a trifling elevation of the snout (36, 42).

It would appear, therefore, that a mere breaking of the fiber which is, for any reason, not followed by retraction is unlikely to evoke a reaction and, presumably, does not disorganize the mechanism of which Reissner's fiber forms part.

3. A comparison, however, of the records of the experiments with the evidence afforded by the microscopical examination of the preserved material in the case of the thirty individuals composing the third group, suggests that there exists a distinct connection between the reaction manifested and the retraction of the fiber.

Thus in certain cases (e.g., 37, 52, 53, 64, 66) in which the reaction had not been particularly pronounced or prolonged, there was found to have occurred a comparatively slight retraction. On the other hand, in a number of experiments (10, 20, 22, 24, 35, 43, 54, 56, 57, 58, 59, 60, 67, 68) in which the reaction had been particularly marked there was found to have

occurred an extensive withdrawal of the fiber, forward, from the region of the experimental lesion.

In a couple of instances (47, 64) this relation is less evident, there having been a somewhat pronounced reaction although the fiber had not been very greatly retracted. Both of these specimens were the subjects of experiments of quite short duration ($1\frac{1}{4}$ hours and 10 minutes respectively) and it is probable that the fiber would have continued to retract had the experiments been allowed to proceed for a longer period.

Reissner's fiber, in three specimens (5, 7, 24), all of which were the subjects of experiments of prolonged duration, is found to extend backwards nearly or quite to the region of the experimental incision.

A secondary sinus terminalis is seen in the process of formation in the last of these and in this case Reissner's fiber has become of almost normal diameter but lies freely with a somewhat fibrillated ending in this new terminal enlargement of the central canal.

The sections through the tails of two other specimens were cut transversely and the condition of the end of the fiber can not be certainly determined. In one (7) the fiber has a diameter but slightly greater than the normal, while in the second (5) it is quite distinctly swollen.

Another specimen (6) is apparently a normal case in which there has been a considerable retraction. The terminal piece sectioned shows that the fiber had withdrawn wholly from that region. The penultimate piece, however, contains the free end of the fiber lying slackly and of quite notable slenderness. I suspect that this may be an early phase of regeneration in which a delicate new growth of fiber is stretching backward, the usual simple straightening out of the original thread having, for some reason, been prevented.

4. Regeneration is seen in a well advanced condition in but a single specimen (2), a dogfish, of which the condition of the end of Reissner's fiber is seen in figure 31. In front of the incision, a secondary sinus terminalis has arisen, the pia mater having grown around the end of the filum terminale where it

was severed to form the delicate hinder wall to this new terminal chamber. The fiber seems to have flared out into a terminal plug in which several strands, one somewhat thicker than the normal fiber, can be distinguished. This lies in contact with the meningeal wall of this secondary sinus terminalis and was either just about to become attached to the meninges when the specimen was killed or, more probably, had actually made its new terminal attachment.

5. It will now be convenient to consider more fully the condition of Reissner's fiber in the subjects of eight experiments (3, 9, 19, 39, 46, 49, 55 and 70) which I have refrained from including in either of the four groups, although concerning most of them I have but little doubt as to which category they really belong.

Thus in the case of no. 39 which was an experiment of quite short duration, the sections show that, although broken by the experimental incision, the fiber has not retracted forward from the incision. Since the ray exhibited no reaction after the operation, it is clear that we have a specimen which should be placed in the second of my four groups. During the dissection, however, a slip of the knife inflicted a cut far forward in the spinal cord. As the result of this post-mortem injury, a retraction of the fiber took place from before backwards and a simple spiral twisting has been produced which has affected the fiber back to the region of the incision.

A similar accident occurred to no. 9 with a similar effect upon the fiber. In this case, however, the experiment had been one of considerable duration (11 days) and there had been manifested a well marked reaction. It is extremely probable, therefore, that in this specimen there had resulted the usual considerable retraction of the fiber which had, however, become straightened out before the specimen was killed. Reissner's fiber is found in the sections extending fully to the point where the filum terminale had been severed and, in this resembling the condition of the fiber in no. 39, it is found twisted into a nearly continuous simple spiral (text-fig. 2). There can be little doubt that, but for the accidental breaking of the fiber after the death of the

animal, the experiment would have been found to belong to the third of my four classes.

The case of no. 19 is of a different kind. In this specimen no reaction appeared as the result of the operation yet, in the sections, the severed end of the fiber in front of the lesion was found to be retracted for a short distance, swollen and, near its free end, spirally coiled. The latter detail probably affords the clue to what might, in view of the absence of any reaction, appear as a distinct anomaly. The experiment had continued for 6 days and, therefore, if there had taken place a retraction of the fiber so extensive that the fiber had not straightened out in that time, a well marked reaction should have been evident. Spiral coiling, however, in every other instance known to me, is associated, as I shall show, with recent retraction. In this instance, then, there can be little doubt, I think, that the specimen was one which would in the ordinary way have been included in the second group—i.e., among those in which the fiber was severed but failed to retract—the severed end being gripped, probably, by the compression of the walls of the filum terminale. During the handling which is unavoidable where a rapid dissection is desired, the fiber may have been released and then have commenced to withdraw. A disturbance which freed the fiber from the grip of the walls of the filum terminale doubtless afforded, at the same time, ready ingress to the fixing fluid and thus quickly checked the incipient retraction.

In the last experiment performed (70) there was again an apparent discrepancy. This specimen on recovering from the anaesthetic, assumed a quite unusual position (fig. 10) which appeared to be an obvious reaction to the experiment. Subsequent examination of the material under the microscope revealed, however, that the experimental incision had just failed to cut the filum terminale. As it happens, the greater part of the filum terminale in the piece of tissue sectioned is contained in one thick section in which, although the presence of Reissner's fiber can be ascertained, it is not possible to make out its condition. Owing to a slight distortion of the material, however, the sinus terminalis lies in an adjacent section which is moder-

ately thin. From this terminal region the fiber is certainly absent; the sinus terminalis itself shows signs of disturbance and contains the remains of a clot which is certainly not the result of the experimental incision, which did not destroy the pia mater.

The specimen must have been one which had been brought in recently, probably the previous day, for during my stay at Plymouth I made use of all the moderately small specimens which were available within a very short time of their capture. Since in this specimen, the fiber is absent from the region of the sinus terminalis and the latter chamber itself is somewhat disrupted (for which injury my experiment was not responsible) it is almost certain that the ray had been damaged in the trawl, probably on the previous day.

That there was no reaction in evidence when the specimen was selected for the experiment is doubtless to be explained by the fact that the reaction frequently appears intermittently. Moreover, the experiment was the last undertaken and was performed in some haste so that the usual precaution of keeping the specimen under observation for some hours prior to the operation was not taken in this instance.

The reaction seen in this experiment, therefore, is almost certainly to be attributed to an accidental breaking of the fiber at some time prior to the experiment and is in no way due to the experimental incision which did not disturb the central nervous system.

Two other specimens (3, 49) reveal the fiber broken as the result of some accident, but in these cases the snapping of the fiber must have taken place in the trunk (or head) region and must have occurred only a very short time indeed before the operation.

In the case of the former (no. 3, the subject of the first experiment performed upon a ray) an overdose of chloroform was administered and for some 3 hours following the operation there was no sign of returning animation. At the end of that time it was decided to discontinue the experiment and the central nervous system was exposed and hardened. As it happened, this experiment, while affording no information upon the function

of the fiber, provided material which throws considerable light upon the recoil of the fiber.

A general account has been given above of the condition of Reissner's fiber in the hinder part of the spinal cord of this specimen. From that description it will be apparent that, since both before and behind the incision the fiber extends actually to the severed ends of the filum terminale (text-fig. 4), there could have been no retraction of the fiber as a sequel to the operation. Nevertheless, in the condition of the fiber, both in front and behind the point where it was broken by the operation, there is very distinct evidence of a recent retraction.

In the terminal (severed) portion of the terminal filament (text-fig. 4 a) the fiber is seen to be considerably swollen, the swelling becoming more pronounced in the terminal sinus where the fiber passes into a loose spiral; the terminal plug is not recognizable, having collapsed, presumably, when the recoil began.

Immediately in front of the incision (text-fig. 4 b) the fiber is found in a wonderfully twisted state and continues markedly coiled to the forward end of the piece of tissue examined. The torsion is not uniform, short simply-twisted stretches intervening between greatly convoluted lengths of fiber. As already pointed out, this short length of terminal filament contains very many times its length of Reissner's fiber. It is obvious, then, that not only must this retraction have been due to a withdrawal of the fiber from before backwards but, also, that it must have been in progress prior to the operation, since otherwise it could not have affected the severed piece of fiber in the region behind the experimental lesion.

The incipient coiling of the fiber behind the incision is evidence that retraction had been in progress but for a very short time when the experimental incision separated this terminal portion of the fiber and, as it happened, checked further recoil behind this point. In front of the lesion, however, a gradual retraction continued during the 3 hours while the specimen lay motionless,[3] until a great length of Reissner's fiber had accumulated in the hinder part of the spinal cord.

[3] Doubtless the retraction actually continued until finally stopped by the hardening action of the fixing fluid.

That the fiber was broken just prior to the experiment, in no. 49, also, appears extremely probable. In the trunk region the central canal of the spinal cord is found empty of fiber, while in the hinder part of the spinal cord and the filum terminale the fiber is seen much coiled and swollen throughout the entire length of two pieces of the tail region which were sectioned. Here, too, as in no. 3, there was practically no retraction forward from the lesion (fig. 22) so that the whole of the retraction observed must have resulted from the backward withdrawal of the fiber towards the tail. Behind the incision the fiber extends to the severed end of the terminal filament but is loose and undulating, shows some spiral winding and, near the terminal sinus, is distinctly swollen. As in the previous case, therefore, we have the evidence of a retraction which has started prior to the operation and was the result of an accidental breakage of the fiber far forward in the trunk region. In this case the specimen recovered from the anaesthetic and manifested a marked reaction, not to be attributed to the experimental incision. The experiment, however, was of much shorter duration than was the case in no. 3 and the less intricately coiled condition of the fiber in this specimen is clearly related to the shorter period during which the fiber was free to withdraw. In nos. 9 and 39, in which the accidental cutting of the fiber took place after death, the fiber was free to retract only for the much shorter period which was required for the penetration of the fixing fluids. In both of these specimens the fiber has simply undergone a fairly regular twisting but has not produced the more complicated secondary spirals seen in no. 49 and, still better developed, in no. 3.

In both of the two experiments which remain to be considered (nos. 46 and 55) the fiber appears as an extraordinary delicate filament lying somewhat slackly but not apparently withdrawn from the injured place. In neither case was the experiment of long duration and in both the reaction observed took on a somewhat unusual character, there being manifested a distinct departure from the normal pose but no appreciable deviation of the long axis from the regular straight line. While this reaction

was not one which I should be inclined to describe as 'marked' it was, nevertheless, too considerable to be attributed to the scarcely appreciable retraction which has occurred at the severed end of the fiber.

If, then, I am correct in regarding the occurrence of this exceptionally delicate fiber as indicative of an early stage in a new backward growth of the fiber after some unusually extensive retraction, the reaction noticed in these two experiments may perhaps have been the consequence of a renewed disturbance of the Reissner's fiber mechanism in specimens in which the repair of a previous disturbance had scarcely been completed.

The condition of Reissner's fiber in the subjects of these eight experiments may therefore be summed up as follows.

One (19) is to be regarded as exhibiting a slight retraction of the fiber started at the moment of fixation of the material and quickly checked; two others (9, 39) showed a considerable retraction resulting from an accidental cutting of the fiber during the dissection made to expose the central nervous system. The remainder are regarded as showing stages in the retraction (or repair) of the fiber consequent upon a breaking of the fiber prior to the experiment. This snapping of the fiber may have occurred immediately (3, 49) or some little time (70) or some considerable time (46, 55) before the incision was made. That such a breakage of the fiber does occur not infrequently in life and that it may produce a reaction comparable to that induced by artificial section of the fiber will be seen from the account given in the following section.

2. Non-experimental material

An attitude similar to that induced in many specimens by the experimental incision, was occasionally noticed in specimens (not the subjects of the experiments) confined in the aquarium of the Plymouth Biological Station.

Of these, one—a dogfish (F)—was obtained during the summer of 1910. It had been seen in the aquarium at intervals extending over several days with both head and tail well up.

was pointed out that in the single example (8) in which the fiber, although broken, had failed to retract there had been no marked reaction.

These conclusions, based upon the examination of material from a comparatively small number of experiments, are strongly supported by the results of the much more numerous experiments which were subsequently performed. The examination of the condition of Reissner's fiber in several specimens which were not the subjects of experiment has provided further evidence in corroboration of the correctness of those conclusions.

The results of this investigation may be said, therefore, to afford very definite confirmation of Dendy's suggestion (put forward in 1909) that the fiber forms part of a mechanism which is concerned in the automatic regulation of the flexure of the body. This hypothesis is quite in harmony, moreover, with certain observations recorded by Sargent ('04), although that author interpreted the facts in an altogether different sense (vide infra).

I have been unable, however, to determine whether the reaction (the assumption of an unnatural attitude at rest and an abnormal action whilst in motion) is to be regarded as the consequence of the diminution of tension at the sub-commissural organ due to the slackening of the fiber or whether it is to be attributed to the putting out of action of a larger or smaller number of the scattered sensory cells situated in the epithelium of the central canal.

It has been pointed out that, although there has been found, in some cases, a quite considerable retraction of the fiber in the hinder part of the spinal cord, accompanied by much swelling and spiral winding, yet in the anterior region of the spinal cord and in the brain itself the fiber may appear to be practically normal. In such a case it seems improbable that any appreciable diminution in the tension of the fiber could have been felt in the region of the sub-commissural organ. Further, in those examples in which the slackness of the fiber has extended far forward, even though it be not accompanied by swelling, it is inconceivable that it could have taken place without rupturing a

great number of those delicate component fibrillae (as I believe them to be) which serve to support and stay the fiber along the length of the spinal cord.

In the subject of two of the experiments (3, 49) the fiber had broken very far forward; of these, one failed to recover consciousness and gave no reaction, but it is extremely significant that in the other (49) the reaction took on a somewhat peculiar form. It is suggested, therefore, that in this case (where the breaking of the fiber must certainly have reacted upon the subcommissural organ), the more pronounced reaction was the sequel of an unusually extensive disorganization of the apparatus.

In this connection, it is interesting to recall what has been recorded by Sargent concerning his experiments. That author laid much stress upon the fact that the subjects of his experiments would blunder, headlong, into obstacles (stationary or other). This behavior, as I have already pointed out ('12, p. 420), is to be noted in the lesser dogfish both in normal (control) specimens as well as in the subjects of the experiments when removed from the comparatively spacious tanks of the aquarium to the smaller tanks in which, alone, one can be certain of keeping them under close observation. Moreover, this blundering gait disappeared, after a few days confinement in the more limited space, in the subjects of the experiments as well as in the control specimens.

In the larger sharks of which Sargent made use, and which were apparently freshly caught specimens, one cannot wonder at such a result. Moreover, Sargent had no opportunity to observe the passing of this phase, for his specimens after a day or so became quite lethargic and died upon the fourth or fifth day of the experiment. Nor does the failure of the fish to avoid collision with the walls of its cage bear out Sargent's contention that there was in these specimens, a delay in the transmission of the optical stimulus, for such an object, always present, would be visible for a sufficiently long period to allow any optical stimulus to pass by the ordinary conduction paths. Even where an obstacle might be interposed with extreme suddenness it would have been scarcely possible to observe any delay

2. The spiral winding of the fiber and the occurrence of 'snarls'

Examples of the peculiar spiral contraction of the fiber have been seen in a number of the experiments. Thus numbers 3, 4, 9, 19, 34, 35, 37, 41, 49 and 56 all show the fiber twisted to a greater or less extent. That the list is not more lengthy is to be explained by the fact that in a number of cases I have not cut sections of the spinal cord sufficiently far forward to find the retracted end.

There is distinct evidence that, in the case of no. 3, this retraction (though not the result of the experimental incision) must have taken place, for the most part, during the three hours or so which elapsed between the operation and the fixation of the material. The similar but less extensive coiling which occurred in no. 49 (again not the result of the experimental incision) must, likewise, have been produced almost wholly after the fiber was severed by the operation, for the severed portion of the fiber behind the incision is but little affected. This specimen was allowed to live but an hour and a half after the beginning of the experiment and there is doubtless a connection between the less intricately coiled condition of the fiber in this specimen and the shorter period which elapsed between the breaking of the fiber and fixation.

The case of no. 9 differs from that of the two preceding specimens in that the cut which started the backward recoil was made after the death of the specimen just as the material was about to be plunged into the fixing fluid. The simple and continuous spiral winding which is found in this specimen can have been produced, therefore, only during the time which was necessary for the fixing fluid to thoroughly penetrate and harden the material.

In the case of experiments 34 and 56 (both of which were of short duration) the fiber has retracted (spirally) away from the lesion and the recoil, therefore, was definitely a consequence of the experimental incision. The character of the spiral winding in the fiber in the case of four other experiments (4, 35, 37, 41) is somewhat different. It is found at the free end but does not

extend for a great distance along the fiber and has a much looser twist which suggests the uncoiling of a spirally twisted thread. All four of these experiments had a relatively considerable duration, the subject being killed towards the end of the first day or during the second.

Apart from experiment 9 which was vitiated subsequently (by an accidental cut during dissection), the only case in which even a slight spiral twisting was observed in an experiment of long duration is no. 19. The subject was a ray, which, during the whole time (nearly 7 days) which elapsed between the breaking of the fiber and the killing of the specimen, gave absolutely no reaction. I have already suggested that it is probable we are dealing, in this case, with a slipping and coiling of the fiber which was started during the dissection by a reopening of the wound made by the operation but was quickly checked by the rapid penetration of the fixing fluid.

With but this single possible exception, therefore, every case of spiral winding of the fiber has been found in the early stages of the experiment or immediately following an accidental cut; by the second day this torsion has usually disappeared and, where it is found at so late a period, is almost certainly in process of uncoiling.

In confirmation of the view that the spiral coiling is found as the result of a comparatively recent snapping of the fiber, it may be noted that, while it has been found frequently in material in which the spinal cord has been severed during or immediately prior to fixation, it is more rarely found in specimens of which the nervous system had been preserved entire.

In many larval lampreys and in adult myxinoids, all of which were preserved entire, I found, it is true, an intricately coiled mass of fiber which, in some cases, almost fills the sinus terminalis[4] ('12 a, figs. 15, 17, 18). In none of these specimens had there been any attempt to expose the central nervous system

[4] That this terminal coiled mass, though so frequently found, is not, as Studnička ('99) supposed, the normal condition is proved by the fact that Sanders has seen a taut condition of the fiber in the sinus terminalis ('94, p. 11) comparable to that which I have described in the lamprey and other forms ('12, '12 a).

and fixation, therefore, had necessarily been slow. I have suggested ('12 a, p. 27) that unusually strenuous exertions made by the animals in their unavailing efforts to avoid capture may be the cause of the somewhat frequent instances of broken fiber noted in these cyclostomes.[5] Careless handling of the specimens might be equally responsible for the snapping of the fiber. It is possible, therefore, that the apparent absence of the fiber from the spinal cord of specimens which have been some time dead before fixation may not be due (as I have supposed) simply to the degeneration of the fiber having already occurred, but may be owing rather to its having been broken and retracted entirely beyond the limits of the piece or pieces of tissue examined.

In two rays which, although not the subjects of experiments, manifested a well marked reaction a spirally coiled condition was discovered in the broken fiber. One (Raia XIX) is known certainly to have been taken some 24 hours before the material was preserved and it is probable that a similar interval had elapsed between the capture and preservation of the material in the case of the second (XXXIX) also. The former bore signs of recent damage in the tail region evidently caused by the trawl but the other appeared externally to be undamaged. If, therefore, the fiber had been broken at the time of capture, in consequence of the violence of the animal's struggles to escape, the fiber might be expected to have become straightened out or to be in the process of unwinding. In one (XXXIX) the free end of the fiber is loosely curled (fig. 20) and in the other (XIX) a tangle remains as evidence of a sharp recoil, but the spiral winding is found only in the vicinity of the tangle (fig. 25) and has, elsewhere, disappeared.

That the fiber was liable, in its recoil, to form intricately tangled knots or 'snarls' was first noticed by Sargent ('04, fig. S). His figure does not suggest the spiral winding which I believe to be associated with the recent contraction of the fiber and which is seen in the photomicrograph which I published in 1912 ('12, fig. 3).

[5] The breaking of the fiber, prior to the experiment, in nos. 3 and 49 is probably to be attributed to this cause.

At the commencement of this investigation I inclined to the idea that such a knot would be invariably produced when the broken fiber retracted, and supposed that the spiral winding, extending more or less uniformly along a great length, would be found only in those cases where a gradual process of fixation prevented the more sudden recoil. The results obtained from a large number of experiments indicate, however, that this sudden contraction may be of much less general occurrence than was supposed and that the withdrawal of the fiber is brought about usually, by the simple spiral twisting of the fiber.

The tightly knotted tangle of fiber present in Raia XIX, just above mentioned, is the only example of this condition which I have encountered in the course of this investigation. There is reason to believe that in this case the fiber may have broken some 24 hours before the material was preserved. In another specimen (10) in which the fiber was broken by the experimental incision made some 10 days before the fixation of the material, there is found a loosely twisted skein of fiber, a small part of which is represented in figure 26. That this condition had been preceded by the tightly knotted condition is very probable, this knot having doubtless served to hold the broken end and thus prevent more extensive retraction, for the tangle is found at no great distance (about half an inch) from the point of injury. It must be supposed, therefore, that during the 10 days of the experiment the spiral torsion had disappeared and the process of disentangling the snarl had been proceeding. The fiber, except at its immediate hinder end, had become nearly normal in size, but whether the tangle would have been smoothed out eventually, if the experiment had been prolonged, or whether a new delicate growth from the hinder free end would have followed, I have no evidence to decide.

8. The duration of the reaction and the problem of regeneration

It is not quite obvious why the reaction appears to be so variable in its duration. If the assumption of an abnormal attitude in repose is, as I believe, a consequence of the disorganization of the mechanism of which Reissner's fiber forms part, we

should expect that the reaction would continue until the fiber had reëstablished its attachment to the walls of the sinus terminalis and had once more attained to its normal tension.

The reaction did persist, indeed, in some specimens until the attachment was practically made good (2) or until the termination of the experiment (9). Occasionally the reaction was manifested intermittently for several days (5, 6) while in one case (24) it reappeared after several days of apparent normality. On the other hand, a reaction was sometimes marked during the early hours of an experiment but was not noticed subsequently (7) although the sections showed that the fiber had been broken but gave no indication that the new terminal attachment was completed.

While, then, it is quite possible that in some specimens (in which the reaction had seemingly disappeared and which were killed very soon after the operation) the reaction might have reappeared at intervals had the experiment been prolonged, it is probable that in many the reaction would have apparently completely vanished (as in 7).

Nevertheless, it seems unlikely that the effect produced by the breaking and retraction of the fiber can really altogether disappear until the tension of the fiber has been restored.

The more obvious irregularities of the pose are possibly soon corrected, to a large extent, by the aid of the other senses, notably that of touch, and these corrections would be likely to become more exact as time passed, thus accounting, in some measure, for the gradual diminution in the magnitude of the reaction. It is, however, extremely probable that there may have been other reactions which persisted long after the specimens seemed to me to be normal. Especially may this have been the case with minute irregularities in action, in swimming, for whilst in motion the correcting influence of the sense of touch, at least, would almost certainly be eliminated. The motion of the animal is particularly difficult to observe closely and defied my attempts at analysis so that, although at times I felt convinced that the action was not exactly that which is

usual, yet it was extremely difficult to decide wherein the difference lay.

There is yet another possible explanation. It has been seen that, where retraction did not follow the breakage of the fiber, there was no obvious reaction. It has been assumed that this was due to the maintenance of the tension of the fiber by the firm grip of the adpressed walls of the filum terminale upon the severed end of the fiber. Not only, however, might the tension be maintained but the connections with the numerous sensory cells in the central canal, whose filamentous processes contribute to the substance of the fiber, are preserved intact. The absence of the reaction may be partly or wholly attributable to this latter fact for, although the terminal plug at the hinder end of the fiber is clearly to be regarded as the principal insertion of the fiber, yet there can be little doubt that the attachments of the fiber by the component fibrillae throughout the length of the spinal cord must afford a very considerable support. Such evidence as these experiments have afforded suggests that the greater the extent of the retraction forward the greater is the degree of the reaction. It may well be, then, that as soon as the forward retraction is checked and the repairing process has brought about the unwinding and straightening out of the fiber, the component fibrillae may forthwith begin to renew their attachment to the fiber. In this way while they may assist in restoring the tense condition of the whole fiber a constantly increasing number of sensory cells may be coming into action again, the diminution of the visible reaction being attributable to the restoration of these connections.

Sargent has stated ('04, p. 230) that "sharks have shown almost no capacity to heal wounds or regenerate skin." During the progress of this investigation several rays were taken in which there was evidence of the loss of part of the tail but the stump had healed perfectly. While it may be that, as regards this power of regeneration, rays differ from sharks and dogfish, it must be remembered that my rays were, in general, quite small specimens and it is exceedingly likely that the injury had been inflicted when the specimens were very small, indeed.

In young animals the recuperative powers are frequently much greater than in aged specimens and it may prove that the restoration of the normal (functional) condition of Reissner's fiber after injury may be effected much more quickly in some specimens than in others.

In this connection the condition of Reissner's fiber in the dogfish (F) is of interest. This specimen, it will be remembered, was one which was seen in the large aquarium tank exhibiting, very markedly, the reaction which is associated with the broken and retracted fiber. The fish had certainly been in confinement for some time and there was reason for connecting the injury to the hinder border of the caudal fin with damage inflicted by the trawl. The injury was, therefore, probably of long standing and the reaction had almost certainly persisted for a considerable time. In the sections the fiber was found to be somewhat slack, lying near its free end in loose undulations and there were no indications that regenerative processes were at work. The specimen was unusually large and presumably an old individual. There seems, therefore, to be in this case a connection between the size (age) of the specimen, the lack of regenerative powers and the continuance of the reaction.

As already noted, I have but one example of undoubted repair of the mechanism (fig. 31) after the experiment. This is seen in a dogfish (2) which was killed sixteen days after the operation.

In several cases, however, the fiber had clearly undergone a considerable retraction but had straightened out again before the specimens were killed. In the sections, therefore, it is seen to extend almost or quite to the spot where it had been broken by the experimental incision. Examples of this phase of repair are to be seen in nos. 5, 7, 8 and 24. The disentanglement of the snarl (10) and the unwinding of the spirally twisted fiber (37, 41) must be regarded as preliminary stages in the process of repair. In no. 18, where there had been no retraction, it would seem as though the end of the fiber was flaring out in preparation for a new terminal plug (fig. 28).

Whether the delicate fiber found extending backward slackly in some specimens (46, 55) almost to the region of the incision, is to be regarded as yet another early stage in regeneration is less certain. Possibly it is a backward growth from the free end of the fiber in a snarl which has altogether failed to become disentangled. On the other hand the fineness of the fiber may be nothing but an individual peculiarity.

It is noteworthy that, where this abnormally fine fiber was found, it had retracted but little when cut and the consequent reaction had proved to be but slight.

In view of the fact that a case of complete repair had been obtained in but a single experiment (2), it has not been possible to determine the period within which regeneration might normally be expected to take place. If the incision has completely divided the filum terminale (in practice an inevitable consequence of any attempt to cut the fiber) even though this be very far posteriorly, it is almost certain that regeneration cannot be effected until a new (secondary) sinus terminalis has been formed. It is unlikely that this comes into existence earlier than the end of the second week, although the exact time would depend upon the regenerative powers of the tissue of the individual and would be likely to take place more quickly in young and rapidly growing specimens. The new attachment of the fiber may, however, be even then delayed if the retraction has been very considerable, has resulted in a tangled knot, or if the fiber has broken very far forward.

In several of my experiments the fiber had returned nearly to its normal diameter and had pushed backwards to the region of the incision within a week of the operation, while the spiral twist appears to be straightened out during the second day under ordinary circumstances. A complicated tangle evidently requires a considerable period in which to become resolved and in such a knot it is probable that the spiral twisting may persist rather longer. In my experiments, however, only a short length of the fiber was actually separated and consequently no great length of new growth, if any, was required to enable the fiber to extend to the newly formed sinus terminalis. Where,

PLATE 1

EXPLANATION OF FIGURES

1 Scyllium canicula. A photograph of a normal specimen upon which no experiment had been performed. The lower border of the caudal fin is seen lightly resting upon the floor of the tank.

2 A photograph of the subject of experiment 22, taken nearly five hours after the operation, showing a moderate reaction. *, indicates the region of the incision.

3 A photograph of the same dogfish, taken half an hour later, the tail being rather more lifted.

4 A photograph of the subject of experiment 34, taken about two hours after the operation, showing a well marked reaction.

5 A photograph of the subject of experiment 24, taken upon the third day of the experiment, and showing a well marked reaction of the tail.

6 A photograph of the subject of experiment 23, the specimen being seen reposing in the attitude adopted by normal dogfish. The photograph was taken upon the fourth day of the experiment, no reaction having appeared.

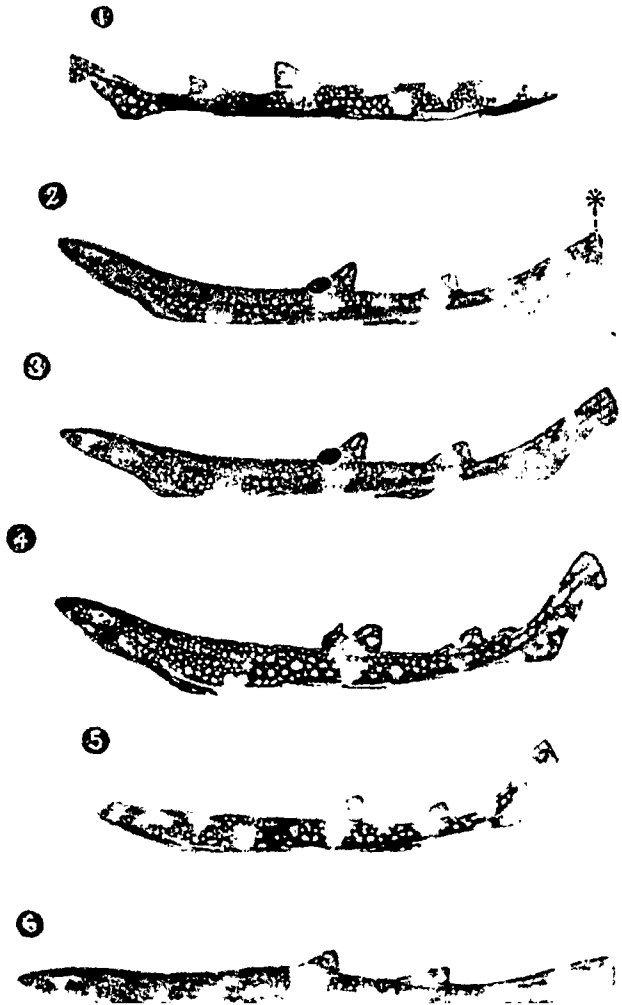

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PLATE 2

EXPLANATION OF FIGURES

7 Photograph of a normal ray (not the subject of an experiment), showing in side view, the attitude of repose which is normal in these animals.

8 A photograph of the subject of experiment 31, showing a reaction affecting the tail only. *, indicates the region of the incision.

9 A photograph of the subject of experiment 68, taken about two hours after the operation. (Cf. figure 13, a photograph of the same specimen taken half an hour earlier.)

10 A photograph of a normal ray (not the subject of an experiment), showing the natural position of the snout in the ray when at rest.

11 A photograph, taken several hours after the operation, of the subject of experiment 30. This ray was one which, while it occasionally showed the typical reaction, rested for the most part in the attitude shown.

12 A photograph, taken a quarter of an hour after the operation, of the subject of experiment 70. The head is seen well raised but the tail appears unaffected.

13 A photograph of the subject of experiment 68, taken an hour and a half after the operation. The tail is seen, somewhat indistinctly, well raised and turned to the left.

14 A photograph of the subject of experiment 56, taken half an hour after the operation, showing an extreme reaction, the body being raised completely from the floor and supported only upon the lateral border of the pectoral fins.

15 A photograph of a ray (XXXIII), not the subject of an experiment, but in the attitude which results from the breaking and retraction of Reissner's fiber. This photograph was taken after the ray had been kept for four days under observation.

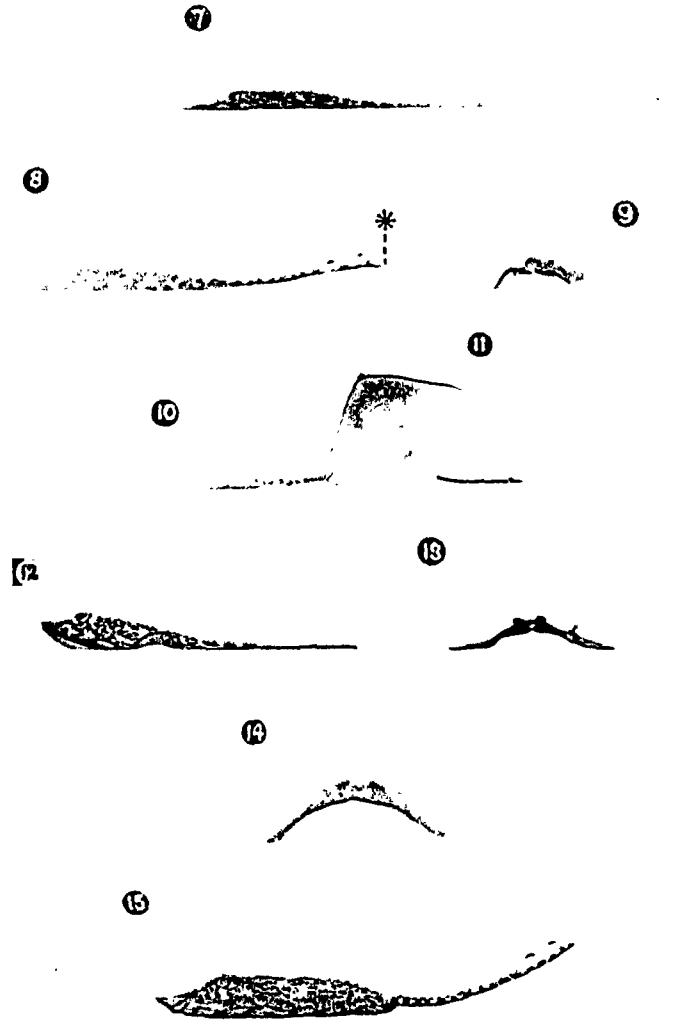
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PLATE 3

EXPLANATION OF FIGURES

16 Raia blanda (49). Part of a sagittal section through the filum terminale showing the free end of Reissner's fiber in front of the lesion (the posterior end to the left, in the figure). × 240.

17 Part of a transverse section through the spinal cord of the cat, showing a much swollen Reissner's fiber, nearly 10 μ in diameter, embedded in a mass of coagulum which almost blocks the central canal. In the center of the fiber can be seen the cut end of what appears as an axial thread or core. × 240.

18 Raia blanda (49). Part of a sagittal section through a piece of the spinal cord from the anterior part of the trunk, from which region Reissner's fiber had wholly retracted (caudally). The middle of the canal is occupied by a filmy structure (x) which is probably coagulum. × 240.

19 Raia clavata (XIX, not experimental). Part of a sagittal section through the end of the tail showing the broken end of Reissner's fiber in the (secondary) sinus terminalis. The terminal neural pore is almost choked by a mass of débris and coagulum. × 240.

20 Raia clavata (XXXIX, not experimental). The broken end of Reissner's fiber is seen loosely coiled near the end of the central canal, the anterior piece of fiber depicted having been added from an adjacent section. The (primary) sinus terminalis is fairly typical and extends downwards, in normal fashion, behind the end of the notochord. × 240.

21 Raia blanda (11). A length of Reissner's fiber from the fourth ventricle, to show the brittle condition of the preserved fiber, which has splintered upon the microtome knife. × 240.

22 Raia blanda (49). Part of a sagittal section through the filum terminale, immediately in front of the lesion. Reissner's fiber is seen entangled in a clot from which there has been no apparent retraction forward. Nevertheless, the whole length of fiber in the piece examined is spirally coiled, this being the result of a backward recoil from some point in the spinal cord. × 45.

23 Raia clavata (37). Part of a sagittal section through the filum terminale, in front of the lesion. Reissner's fiber is seen swollen and irregularly coiled. × 240.

ABBREVIATIONS

c.c., central canal of the spinal cord (and terminal filament)
cg., coagulum, practically filling the central canal (fig. 17)
cl., blood clot, in the central canal
e., epithelium lining the central canal
f.t., filum terminale
nch., notochord
R.f., Reissner's fiber
s.t., sinus terminalis
t.n.p., terminal neural pore
v.c., vertebral column
**, indicate the region of the incision

THE FUNCTION OF REISSNER'S FIBER

GEORGE E. NICHOLLS

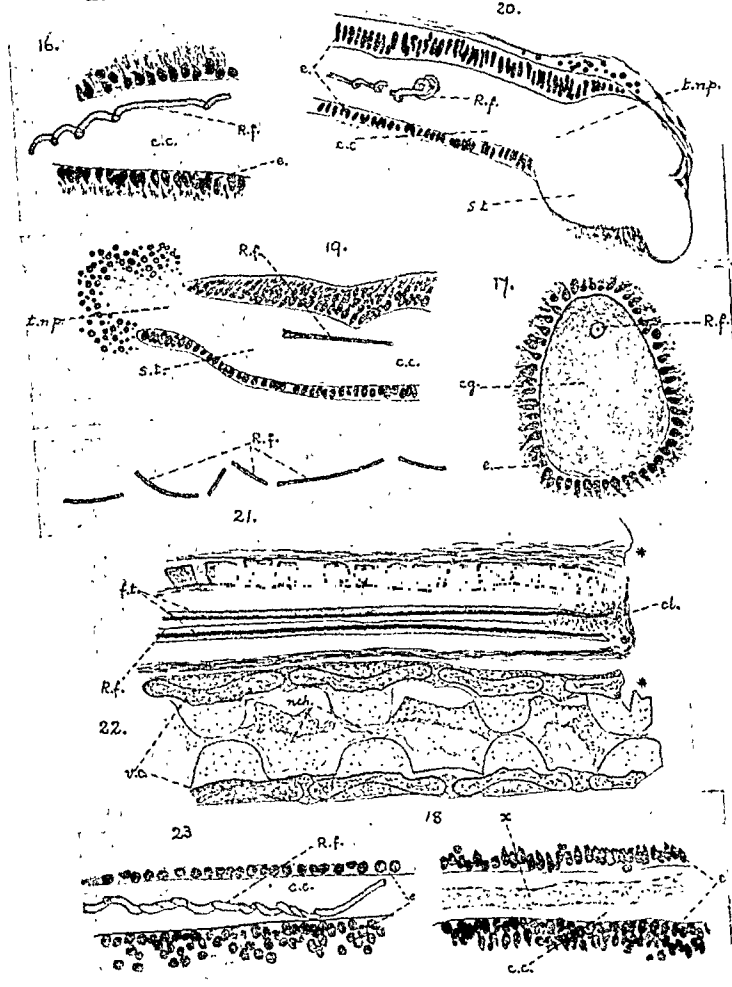


PLATE 4

EXPLANATION OF FIGURES

24 Raia clavata (5). A transverse section (slightly obliquely cut) through the filum terminale, at a point a little in front of the sinus terminalis. Several fibrillae are seen which have apparently broken free from the displaced and slack Reissner's fiber. × 320.

25 Raia clavata (XIX, not experimental). Part of a sagittal section through the filum terminale. Posteriorly the fiber is seen swollen but fairly regular. It passes into a tightly tangled knot, from the anterior end of which it emerges, loosely coiled. (Posterior end to the left, in the figure.) × 240.

26 Raia blanda (10). Part of a sagittal section of the hinder end of the spinal cord, some half inch in front of the lesion. The posterior end of an extensive but loosely tangled skein of Reissner's fiber (of nearly normal diameter) is seen in the central canal which is cut obliquely. (Posterior end to the left in the figure.) × 320.

27 Raia clavata (64). Part of a sagittal section through the filum terminale in front of the lesion. Reissner's fiber is very fine and, to the left (anterior) of the figure is seen in apparent contact with the wall of the central canal. Behind this point it lies slackly, well away from the wall of the canal. × 320.

28 Raia clavata (18). Part of a sagittal section through the filum terminale, immediately in front of the lesion. Retraction of the fiber was prevented by the formation of an extensive clot (lying more anteriorly and not shown in the figure). The severed end of the fiber is seen fibrillated and flaring as though to produce a new terminal plug. × 240.

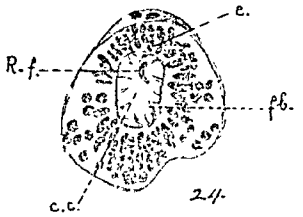
29. Salamandra maculosa. Part of a transverse section through the spinal cord. Reissner's fiber apparently receiving three or four constituent fibrillae. Near the dorsal line there projects a conical process which is probably the apex of a sensory cell. × 340.

30 Scyllium canicula (44). Part of a sagittal section through the filum terminale showing the unretracted fiber, with what are apparently constituent fibrillae in situ. × 340.

31 Scyllium canicula (2). Part of a sagittal section through the filum terminale at the point where this was severed by the experimental incision. The cut end has become rounded off and the meninges have grown around it to completely enclose the secondary sinus terminalis. The end of Reissner's fiber is seen flaring somewhat and has, apparently, made good its new attachment. × 340.

ABBREVIATIONS

c.c., central canal of the spinal cord (and terminal filament)
d.b.v., dorsal blood vessel
e., epithelium lining the central canal
fb., fibrillae of Reissner's fiber in the central canal
f.t., filum terminale
mn., meninges, forming the hinder wall of the sinus terminalis
R.f., Reissner's fiber
s.p., sensory process (?)
s.s.t., secondary sinus terminalis
**, indicate the region of the incision



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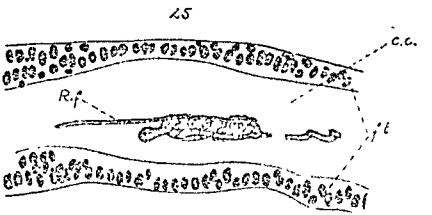

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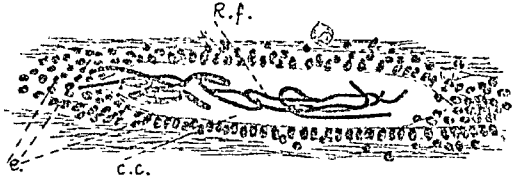

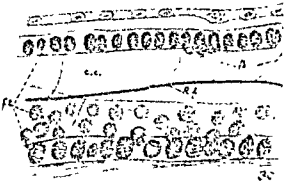

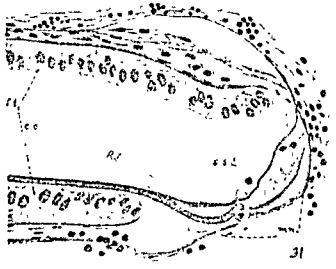

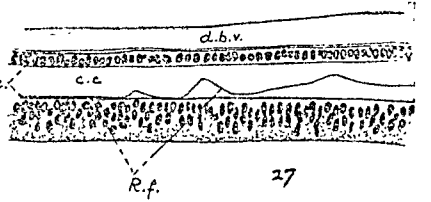

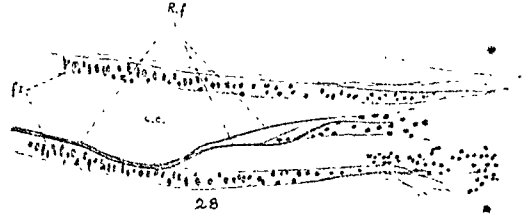

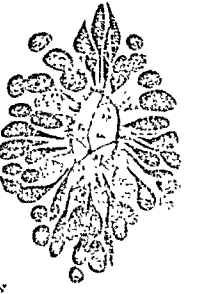

STUDIES ON THE OLFACTORY BULBS OF THE ALBINO RAT—IN TWO PARTS

I. EFFECT OF A DEFECTIVE DIET AND OF EXERCISE

II. NUMBER OF CELLS IN BULB

CAROLINE M. HOLT

From The Wistar Institute of Anatomy and Biology[1]

FOUR PLATES

PART I. EXPERIMENTS TO DETERMINE THE EFFECT OF A DEFECTIVE DIET AND OF EXERCISE UPON THE WEIGHT OF THE OLFACTORY BULBS

CONTENTS

[1] Thesis presented to the Faculty of the Graduate School of University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

I. INTRODUCTION

The various members of the mammalian series show considerable variation in the relative development of all parts of the central nervous system, but probably no part of the encephalon shows so great a degree of variability as does the rhinencephalon. Of this portion of the brain, the olfactory bulbs are, without doubt, the most variable in size. Thus we have the very large bulbs of the opossum and the ant-eater; the almost rudimentary bulbs of the ape and of man; extreme reduction of these organs in the Cetacea, with their complete disappearance in the dolphin. Not only do we find variation in size of the olfactory bulbs among the different orders of mammals, but we find that there is a considerable degree of variability within each order and even among the members of the same species.

This variation in size and weight of the olfactory bulbs within a species is well illustrated by observations upon the rats in the colony of The Wistar Institute. The domesticated albino rats exhibit a considerable range in the development of this part of the brain. But while we find an appreciable difference in the bulb size of rats of different litters even under like environmental conditions, the individuals of a given litter usually show a more uniform development of the olfactory system. Some wild Norway rats examined at The Wistar Institute a few years ago had olfactory bulbs heavier in proportion to total brain weight than the bulbs of the albino. In the course of the present study, observations made upon some thirty wild Norway rats caught at different places in Philadelphia suggested that this difference between the two strains is not a constant one, for,

while the olfactory bulbs of these animals were much heavier than those of the albinos, the ratio between bulb weight and brain weight in this series was about the same in the two forms.

Inequality in the size of the two bulbs in the same individual appears not infrequently in the albino and when it occurs it will often be found in several, and occasionally in all, members of the same litter. For this reason, in selecting material for these experiments, we discarded all litters in which cases of asymmetry were observed among the initial controls.

Observations made from time to time by Dr. Donaldson, indicated that rats born in the early summer differ from winter-born rats in the relative size of the olfactory bulbs; also that there might be a difference between rats reared on a restricted diet, such as is frequently used in colonies, and those fed on the table-scrap diet adopted for the Wistar colony. Moreover, cases had appeared in which the bulbs of sick rats were apparently smaller than those of healthy individuals.

All these facts suggested that there might be factors in the living conditions of the rats which would account for the variability of this portion of the nervous system, the growth of the bulbs being retarded or arrested in rats reared under unfavorable conditions, such as the intense heat of the summer, or a monotonous diet, or in those suffering from the various infections which may attack the rats from time to time.

It was, therefore, with the hope of throwing some light upon the question of the effect of environmental conditions upon the olfactory bulb of the growing albino rat, that, at the suggestion of Dr. Donaldson, the present experiments were undertaken. The problem resolved itself into two questions—Can the growth of the olfactory bulbs of the stock albino be modified (1) by underfeeding or (2) by exercise?

The writer wishes here to express her deep gratitude to Dr. Donaldson for his unfailing helpfulness and encouragement, and her appreciation to Dr. Stotsenburg and Dr. Heuser, and to the other members of The Wistar Institute who did much to aid in the course of the experiments which have extended over the past two years.

ner. The brain was placed, ventral side down, on the dissecting board. Then with a thin, sharp scalpel held in a position perpendicular to the plane of the board and at right angles to the plane of the median longitudinal fissure, the bulb was severed just below the anterior limit of the cerebrum.[2] The bulb, with the remainder of the brain was then placed in a covered weighing bottle and the weight of both the entire brain and of the severed bulb ascertained.

The final controls were weighed and placed under the normal living conditions of the colony: i.e., housed, in long wooden cages with wire fronts, thick shaving-covered floors, and paper nests, and given plenty of fresh water with a carefully supervised scrap diet. The test rats were weighed and placed in adjoining cages under exactly the same conditions as the final controls, save for the diet. The food given the test rats consisted of an unlimited amount of whole corn, usually fed on the cob, save in case of very young animals, or those weak from a long period of underfeeding. In such cases, the corn was shelled as the animals were not able to remove a sufficient amount for themselves.

Both control and test animals were weighed from time to time and the weights recorded. Note was also made of any irregularities, such as a temporary change in diet, etc.

At maturity, a certain number of test and of control animals were mated in order to find out whether underfeeding affected the fertility of albino rats.

In the case of relatively small litters in which the members were usually well grown and in good physical condition when weaned—and especially if weaning was delayed until the rats were four weeks old—it was possible to keep the test animal on a corn diet for a month or more with practically no difficulty.

[2] Small bulbs tend to differ characteristically in shape from large ones. On section it is seen that the cap of gray substance extends somewhat further caudad on the ventral surface of the small bulb than it does in the case of the large bulb. The weight of gray substance thus lost in the case of the small bulb is a very small fraction of the total weight of the bulb but a much larger fraction of the gray cap. Care must, therefore, be taken to include this portion when the number of cells of the gray substance is to be determined.

With the rats weaned at three weeks or in case of small rats from very large litters, there was a good deal of trouble in keeping the animals on the corn diet for so long a time, and of course the difficulty increased as the period of underfeeding was prolonged.

At first an attempt was made to keep animals from several litters in one cage with the result that after a short time, the less well grown rats were killed and eaten by the stronger individuals. Then the plan was adopted of having members of only one litter in a cage. This worked successfully up to the time when the animals began to weaken. Then the males frequently killed and ate the females. So finally, for prolonged experiments, it was found safer to place only animals of the same sex, approximate weight and physical condition together, but even this precaution was not always sufficient.

In most cases, for the first few weeks, there was a very slow gain in weight or the weight was just maintained. But in every case when an animal began to lose or became very feeble, a dose of condensed milk was fed. One or two doses were usually sufficient to restore the animal to equilibrium and there was not infrequently a sudden temporary gain in weight, doubtless due to increased appetite and the consequent gorging of the alimentary tract with corn.

In a few cases where the underfeeding had gone on for several months, it became necessary to administer small doses of condensed milk more frequently—in two cases, practically every day—in order to keep the animals from losing weight.

At the end of the experiment, both test and final control animals were killed, weighed, measured, eviscerated, and brains and bulbs weighed as in the case of the initial controls. One bulb with a part of the cerebrum was preserved for histological study. A record was kept of any signs of disease or other abnormality. The weighing was done in closed bottles and all weights of brain and of olfactory bulbs were made to 0.1 mgm., but recorded here in milligrams only.

b. Results. General morphological and physiological modifications. A summary of the data from observations upon 108

individuals of Series A is given in tables 1 to 8. The complete tables with the records for each individual rat of this, as well as of the other series, are deposited at The Wistar Institute. Of the two litters weaned at eighteen and twenty days, only three individuals survived to be killed; the others died in the cages and the brains were not weighed. The records for the three rats just named have been included in tables 3 and 7, and their controls, with the corresponding controls. The size and body weight of rats weaned at the end of the third week and placed on a corn diet indicated clearly that under like conditions, rats weaned at three weeks are considerably more sensitive to adverse conditions than are those weaned at four weeks.

For every individual of Series A_1 and A_2 (tables 1 to 8), the stunting effect of the corn diet was apparent almost from the first. During the early weeks of underfeeding the test rats appeared rather more lively than the controls. Later this activity decreased, the gait became unsteady, and the animals appeared stupid. They were often unable to find the dish of condensed milk by themselves, whereas control rats would go to it immediately. This suggests that the underfed animals lacked an acute sense of smell and perhaps did not see clearly.

In every one of the test animals of which there are complete records, the general bodily growth was arrested by a diet of corn. This agrees with the observations of Osborne and Mendel ('13). These rats remained like young animals in appearance as well as in size. The earlier weaning took place and the corn diet was begun, the more complete the stunting.

The skeleton became modified and somewhat distorted owing to imperfect calcification. The growth of the long bones was not quite so completely arrested as that of the rest of the skeleton. The skull, sternum, and sometimes the ribs, became like parchment. In two cases the pressure of the heart upon the sternum had formed a sort of pocket out of that structure, which appeared like a tumor on the ventral side of the rat. The vertebral column became somewhat bowed, giving to the rat a 'humped' appearance and making it necessary to stretch the animals when measuring body length. One to four months of

underfeeding, following the first month under normal conditions, left the rats but slightly longer (4 to 10 mm.) than the initial controls measured at thirty days. The average increase in weight was in about the same proportion. Compare tables 2 to 8, for body weight and body length.

All the rats showed extreme emaciation but this condition was largely masked by the condition of the coats. The hair remained short and soft, with a fluffiness which gave even to mature rats the appearance of plump young animals. Such emaciation was, of course, accompanied by great muscular weakness. Rats kept for long periods on the defective diet became unable to remove corn from the cobs. They walked with a tottering gait and moved about but little.

The cyanosed condition of these animals was clearly indicated by the blue color of all exposed parts of the body—nose, ears, feet and tail. In protracted cases of underfeeding, a chronic palpitation of the heart developed which increased in violence as time went on. As a result of this, the whole body shook constantly.

All animals kept on corn up to maturity failed to breed or to show any sexual instinct whatever.

Effect on brain and olfactory bulbs (compare tables 1 to 8). In Series A, both A_1 and A_2 show a slight increase in brain weight during the period of underfeeding. Under normal conditions, as the rat grows, the brain becomes relatively lighter in proportion to body weight. In the underfed rats the brain forms practically the same proportion of the total body weight as in the initial control rats (agreeing with Jackson's results ('15)), which of course indicates in the cases where growth has taken place that the brain has not been as much arrested in its development as has the rest of the body.

After four to eight weeks of underfeeding, the rats of Series A_1 and A_2 had olfactory bulbs which, taken together, formed about the same proportion of the total brain weights as did the bulbs of the initial controls of the same series, showing that the relation of these parts of the brains had not been changed during the experiment. But normally the olfactory bulbs grow faster

TABLE 10. SERIES B
Test animals
Stock albinos underfed from birth. Under two months old

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
6 males	24–38	20.1	86	1.114	0.036	3.19	3.06–3.38
4 females	24–53	15.6	79	1.062	0.033	3.08	2.11–3.82
Averages for males and females		18.1	83	1.091	0.034	3.14	

TABLE 11. SERIES B 1.
Test animals
Stock albinos underfed from birth. Over two months old

RATS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS PER CENT OF BRAIN WEIGHT	RANGE
	days	*gm.*	*mm.*	*gm.*	*gm.*		
3 females	77	31.2	102	1.437	0.050	3.48	3.15–3.93

days old when they were killed and examined. The other eight sick rats of this Series C were individuals showing a considerable infection of the lungs, and one of these (No. 20) had, in addition, a large abscess of the liver.

All of these rats were examined in the same way as those of Series A.

a. Results. In the group of sick animals, those with the intestinal infection had, at one hundred and thirty-four days, bulbs which averaged 0.050 gram or 3.02 per cent of the total brain weight (table 12, group 1) while a set of normal individuals of practically the same age gave an average of 0.073 gram or 4.32 per cent of the total brain weight (see table 20, group of females). These results seem especially interesting because here the adverse conditions appeared only after the rats were well grown—eighty days old—and lasted only about ten days.

The remaining two groups of sick rats all had infected lungs and were very old when killed. The two males had bulbs

averaging 0.037 gram, or 2.08 per cent of the total brain weight (table 12, group 2); while for the four females, the bulbs averaged 0.033 gram, 1.89 per cent of the total brain weight (table 12, group 3). For these last two groups there are no data of normal individuals for comparison but the percentage for the bulbs is strikingly low. Some unpublished data in Dr. Donaldson's hands show, however, that while the relative weight of the olfactory bulbs tends to increase up to about one hundred and fifty days of age, in older rats there is a tendency to decrease so that some of this decrease observed in the old sick rats (groups 2 and 3) may be due to normal age changes. But the remarkably small proportional weight of the bulbs here examined is probably due chiefly to the effect of disease.

In this connection may be mentioned two young rats of litter PR (group 2), killed at seventy days. Each had infected lungs. These rats came from parents with infected lungs and had lived since birth in a dark damp cage. One had very small unequal bulbs which were not weighed. The other had bulbs weighing only 0.019 gram or 1.30 per cent of the entire brain weight. This pair of bulbs were the smallest observed in the whole series of experiments. It seems quite evident that the bulbs are abnormal and quite probable that this abnormality is due to disease.

5. Summary and conclusions. Defective diet experiments

1. General bodily growth in the albino rat is arrested by an exclusive ration of corn which constitutes a defective diet (Osborne and Mendel).
   a. The skeleton is poorly calcified and somewhat distorted.
   b. The muscular system is greatly reduced.
   c. The coat has the appearance of that of a young animal.
2. Functional disturbances follow the arrested development.
   a. There is increasing muscular weakness.
   b. An increasing palpitation of the heart.
   c. The animals appear cyanosed.

TABLE 12. SERIES C

Sick animals

Females

Group 1. Three albino rats from a lot of twelve controls for revolving cage experiment. At about eighty days all contracted a severe bowel trouble from which these three recovered.

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	BULBS PER CENT OF BRAIN WEIGHT
	days	*gm.*	*mm.*	*gm.*	*gm.*	
Z_1	135	128.5	165	1.607	0.033	2.03
Y_{22}	135	150.5	180	1.534	0.034	2.24
X_3	134	157.9	182	1.803	0.082	4.55
Average females		145.6	176	1.648	0.050	3.02

Males

Group 2. Two, old, with infected lungs.

PR_1	70	88	148	1.489	0.019	1.30
PR_6	70	90	155	1.538	unequal	
No. 24	old	213	202	1.759	0.050	2.81
No. 69	365	214	195	1.791	0.024	1.35
Average Numbers 24 and 69		213.5	198	1.775	0.037	2.08

Females

Group 3. Infected lungs, old age, and in case of No. 20, bad abscesses on liver.

No. 20	340	143.0	173	1.673	0.027	1.63
No. 21	340	146.1	187	1.751	0.032	1.79
No. 71	370	186.0	186	1.687	0.051	3.01
No. 72	240	218.3	199	1.794	0.021	1.16
Average females		173.4	186	1.726	0.033	1.89

d. The sense organs become dulled after prolonged defective feeding—the animals respond but slowly to stimulations of sound, or light or smell.

e. Defectively fed animals fail to breed.

3. The effect of defective feeding on the brain and olfactory bulbs is less than upon the rest of the body, but is, nevertheless, very marked. The olfactory bulbs are stunted and to a con-

siderably greater degree than is the entire brain. When defective feeding is begun in rats about thirty days of age, the bulbs of rats thus experimentally stunted form about the same percentage of the total brain weight as do the bulbs of rats of the same litters killed at the beginning of the experiment. Whereas, under normal conditions, the bulbs of older rats (up to one hundred and fifty days) are considerably heavier in proportion than those of the young animals. With prolonged defective feeding the proportional weight of the bulbs tends to become slightly greater.

4. Sick animals, especially those with lung infection, show a marked diminution in the relative weight of the olfactory bulbs, accompanied by a certain amount of loss in total brain weight.

III. EXERCISE EXPERIMENTS

1. Previous experiments on the effect of exercise upon the albino rat

Several investigators have worked upon problems connected with the changes in the albino rat occasioned by an increased amount of exercise. J. R. Slonaker in 1907 published observations upon four rats of different ages kept in revolving cages for a short period. In 1912, the same author published an account of further experiments along the same line, and although this time, also, the work was with a small group of rats, yet the experiment was continued during the natural life of the animals. Slonaker was working chiefly upon the problem of normal activity in its relation to age and sex but, incidentally, he made some few observations upon the comparative development of 'exercised' and normal rats. He found that "exercised rats are more active, more alert, and brighter in appearance than the control ones," but that "the control males reach their maximum weight at an earlier age than exercised males, and also greatly excel them" and that "control rats live longer than exercised rats." No observations were made on the effect of exercise upon any of the internal organs.

Donaldson, in 1911, conducted a series of experiments to ascertain the effect of exercise upon the central nervous system

of the albino rat, using the same sort of apparatus—a revolving cage with cyclometer attachment—employed by Slonaker. He found that there was a slight increase in brain weight (2.4 to 2.7 per cent) to be attributed to the effect of exercise. This was what was to be expected in view of the heavier brain to be found in the wild Norway rat. The cord showed no effect. The olfactory bulbs were not weighed separately.

Hatai ('15) published a series of observations based upon his own experiments and upon those of the present writer, showing the rather marked effect of the same exercise conditions upon the weight of the internal organs. In these experiments, the brains of the test animals showed an excess of 4 per cent over the controls with no effect upon the cord.

2. Description of Experiments. Series D and E

As it had thus been demonstrated that the brain of the albino rat could be modified by exercise in the revolving cage, it remained to determine whether, under such conditions, the olfactory bulbs would show a more marked variation than the brain as a whole.

For this work, also, large litters of stock albinos, were chosen. Each litter was weaned and divided into three groups when about thirty-five days old. One group constituted the 'Initial Controls,' and these were killed and examined as in the previous experiments. The second lot, the 'Final Controls,' w[illegible] in cages under the normal living conditions of th[illegible] third group was used for the experiment. E[illegible] animals was placed by itself in a wire re[illegible] had been used by Slonaker, and later by[illegible]

	.032	1.63
	0.051	1.79
	0.021	3.01
1.726	0.033	1.16
		1.89

Each cage was 5 feet in circumferenc[illegible] fastened to the central fixed axis. [illegible]d after prolonged defective suspended so that, theoretically, [illegible]t slowly to stimulations of floor of the cage to eat. Pr[illegible] to avoid this and so escaped [illegible]ail to breed. exercise. feeding on the brain and olfactory

Each cage was provided [illegible]rest of the body, but is, nevertheless, made and recorded six ti[illegible]ory bulbs are stunted and to a con-

ings showed only the activity of the rats when the cage revolved and were therefore incomplete, since some rats learned to play from side to side of the cage and keep it from revolving, while others learned to run up the middle of the sides in such a way as to hold the cage at rest. But most of the rats soon learned to run the cages and appeared to enjoy it.

The rats were fed on the same diet as the controls and all the animals were weighed at intervals of about two weeks.

3. Series D. Rats in revolving cages for thirty days

There were but two litters in this series. One litter was weaned and set aside at thirty-five days of age and the other at forty days. Both litters were subjected to exercise in the revolving cages for a period of only thirty days. All were killed at the end of the thirty days of exercise.

a. Results. The exercised males of these two litters gained more rapidly in both weight and body length than did the controls, while the females fell behind. The superior growth of the test males was sufficient to bring the averages for both males and females up to 113 per cent of the weight of the controls and to 104 per cent of the length (tables 13 and 14).

The records of the activity of Series D were accidentally destroyed, but as these were for a period of but thirty days, [illegible] would be of little value save in adding further evidence experimen[illegible] male rat becomes active sooner than the male. Slonaker was [illegible] average, there is no difference in the absolute in its relation to a[illegible] test rats in Series D from that of the controls, observations upon [illegible]red with the reference table values in The and normal rats. He [illegible]according to the method there suggested more alert, and brighter [illegible]e the bulbs do show, even after this but that "the control mal[illegible]e unusal activity (tables 13 and 14). an earlier age than exercised [illegible]ke up 4.46 per cent of the brain and that "control rats live lo[illegible]ith 4.36 per cent in the controls. observations were made on the eff[illegible] more marked—4.55 per cent internal organs.

Donaldson, in 1911, conducted a [illegible] making a joint average for ascertain the effect of exercise upon t[illegible] tests against 4.32 per cent

TABLE 13. SERIES D

Test animals

Albino rats kept in revolving cages for thirty-three days after weaning

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	*grams*	*mm.*	*grams*	*grams*	
PR_1	70	88	148	1.489	0.019[1]	1.30
T_2	68	131.7	170	1.871	0.079	4.21
T_2	68	140.9	176	1.831	0.085	4.63
PR_3	70	173.2	185	1.784	0.085	4.79
Average males		148.6	177	1.829	0.083	4.55
Females						
T_1	68	102.9	158	1.744	0.079	4.53
PR_2	70	116.0	162	1.653	0.073	4.39
Average females		109.5	160	1.698	0.076	4.46
Average males and females		132.9	170	1.776	0.080	4.51

[1] Lungs infected. Rat undersized in every way, therefore not included in averages (Series C, Sick rats, p. 218).

in controls, the olfactory bulbs of the former being, therefore, 7 per cent heavier than those of the latter.

4. Series E. Rats in revolving cages for ninety-eight to one hundred and three days

The test animals of this group were kept in the revolving cages for fifteen weeks. At the end of that time, three pairs of test animals and one pair of controls were mated (brother to sister in each case). Some digestive trouble appeared in the cages of control rats rather early in the experiment and most of the rats died, while the remaining animals failed to attain a normal growth, so that satisfactory final controls were lacking for this group. But the rest of the test animals and the surviving controls were killed at the end of the fifteen weeks, measured,

TABLE 14. SERIES D

Final controls

Males

ANIMALS	AGE	BODY WEIGHT	BODY LENGTH	BRAIN WEIGHT	OLFACTORY BULBS WEIGHT	OLFACTORY BULBS: PER CENT BRAIN WEIGHT
	days	*grams*	*mm.*	*grams*	*grams*	
PR_3	70	100 7	154	1 636	0 061	3 72
PR_8	70	90 0	155	1 538	Bulbs unequal[1]	
T_4	68	132.4	170	1 802	0 084	4 64
Average males		116.6	162	1 719	0 072	4 20
Females						
T_1	68	110.8	159	1.780	0 081	4 52
T_6	68	115.6	165	1.774	0 077	4 32
PR_7	70	130.5	170	1 701	0 074	4.32
Average females		119 0	165	1 753	0 077	4.39
Average males and females		118.0	164	1 739	0 075	4 32
Test / Control			103.7%	102.1%	106 8%	

[1] Lungs slightly infected. Not included in average.

weighed, examined, and bulbs preserved exactly as in the underfeeding experiments.

The mated animals were kept about one hundred days longer to see whether the exercise of the previous weeks would show any effect upon fertility.

a. Results. General body growth. We find by examination of the records of body weight taken at two-week intervals during the experiment, that up to the time the larger set of control rats fell sick, the exercised animals were gaining less rapidly in weight than were the controls. From the time of the illness, some five weeks after the beginning of the experiment, the control rats fell off in weight, and with a single exception, they never recovered. Litter W escaped the infection and the weight records for the six rats composing it are as follows:

TABLE 15

Record for Litter W, Series E, showing gain in weight for individuals in revolving cages and for controls

	TEST RATS			CONTROL RATS		
	W_6 (m)	W_4 (f)	W_1 (f)	W_2 (m)	W_3 (m)	W_5 (f)
Initial weight....	44 0 g.	40.5 g.	50.5 g.	43.5 g.	50.0 g.	40.5 g.
2 weeks weight	60 4	62 0	65.6	63 2	68.7	69.2
4 weeks weight....	97.0	82.0	103.0	107.0	120.0	89.0
6 weeks weight.....	139.0	124 0	142.2	139.0	148.8	119.0
9 weeks weight....	186 0	142.0	170.5	194 0	212.0	148.0
30 weeks weight.....	210 0	148.0	187.0	205.0	224.0	150.0
Final length	209 mm.	182 mm.	198 mm.	207 mm.	200 mm.	190 mm.

The test rats from Litter W were, on the whole, slightly longer and lighter in weight than the control animals. The majority of individuals in Litter W proved to have abnormal brains—one or both olfactory bulbs being very much undersized. The brains, therefore, could not be used for comparison and the litter was excluded from the tables. For comparison with the rest of the litters of Series E, it was necessary to use other stock litters, as will be described later (tables 18 to 25). The comparisons are not, therefore, of as much value as they would be were the controls from the same litter. On the average we find body length slightly more, and body weight slightly less, in test animals (table 25). I think we may conclude that these results agree in general with those of previous investigators indicating that exercise has but a slight effect, if any, upon either body weight or body length.

The size of the viscera was considerably modified. These results have been incorporated in the report by Hatai ('15).

Activity of exercised animals. These rats showed great individual difference in the amount of activity and in the age at which they became most active (tables 16, 19, 21, 25). In these respects, there was also a considerable difference in litters as shown by the following record.

If we take the record of these same rats for ninety-three days we get an average of 5.76 miles per day for males, and 5.96 miles

TABLE 16

Activity record of rats in revolving cages for one hundred and three days. Series E

ANIMAL	TOTAL MILES	MILES PER DAY	ANIMAL	TOTAL MILES	MILES PER DAY
Y_7M	914 5	8.9	Y_1M	559 7	5 4
Y_8F	770.5	7.5	Z_3M	476 3	4 6
Y_6F	721 0	7 0	X_4M	470 8	4 6
Y_4F	705.3	6 8	X_2F	458 7	4 5
Y_3M	689.0	6 7	Z_6F	457 1	4 4
Z_5M	577 8	5 6	Z_1F	416.6	4 3
Average for males..	614.7	5 96			
Average for females	593 7	5 76			

for females, and if we go back still further we get a still higher average for the females and lower for the males. The males were slow to begin to run the cages. An extreme example, Y_1 of the present series, ran less than 2 miles during the first five weeks in the cage, but became extremely active during the last four or five weeks making a final average of 5.4 miles per day, a record almost equal to the average for the entire lot of males. The females soon learned to run the cages and became very active at an early age. During the last weeks of the experiment, the activity of practically every female in the series was on the decline. I think from a study of all the records it may be concluded that while, in the revolving-cages, the females reach the period of greatest activity earlier than do the males, yet in the long run, the records of a large number of males and females would average about the same.

Possible effect on fertility. There is some indication that the fertility of the albino rat is increased by exercise. In the cases of the three pairs of exercised rats which were mated, the following record of offspring was obtained, together with the record of one control pair.

The average size of litter for normal stock albinos has been found to be between 6 and 7 individuals (Donaldson '15). This is about the average for the control pair, while the averages for the three test pairs is considerably higher—13, 10.5, and 9.

TABLE 25

(Summary) Showing comparative development of the olfactory bulb

Stunted series

TABLE	NO.	HISTORY	AGE	BODY WEIGHT	BODY LENGTH	AVERAGES FOR BRAIN WEIGHT	BULBS WEIGHT	BULBS PER CENT BRAIN WEIGHT	RATIO AVERAGE TEST TO AVERAGE CONTROL IN			AVERAGE MILES PER DAY
									Body length	Brain weight	Bulbs weight	
			days	*grams*	*mm.*	*grams*	*grams*		*per cent*	*per cent*	*per cent*	
II and XVIII	38	Initial control. Underfed and Exercise Experiment	3; 18–20 35; 29–34	40	111	1 400	0.050	3.54				
X	9	Underfed from birth	27–53	18	83	1.091	0.034	3.14				
XI	3	Underfed from birth	77	31	102	1.437	0.050	3.48				
III	26	Underfed 29–32 days	59–62	57	126	1.504	0.053	3.52	77	91	81	
IV	26	Controls for above	59–62	119	164	1.651	0 066	3.99				
V	3	Underfed 49 days	78–83	44	124	1.487	0.050	3.39	69	87	70	
VI	3	Controls for above	78–83	154	180	1.719	0.072	4.16				
VII	13	Underfed 59–130 days	93–160	51	122	1.524	0.058	3.83	63	85	77	
VIII	12	Controls for above	93–160	187	193	1.772	0 076	4.30				
		Sick animals										
XII	3	Bowel infection	134–135	146	176	1.647	0.050	3.02				
XII	8	Infected lungs	70–370	187	190	1.742	0.034	1.96				
		Exercise series										
XIII	6	Revolving cage 30 days	68–70	133	170	1.776	0.080	4.51	103.7	102.1	106.8	
XIV	6	Controls for above	68–70	118	164	1.739	0.075	4.32				
XIX	8	Revolving cage 103 days	134–135	196	192	1.784	0.079	4.41	99	98	103	5.95
XX	8	Controls for above	93–213 (av. 141)	195	194	1.818	0.076	4.20				
XXI	4	Revolving cage 103 days and mating	237	202	214	1.823	0.086	4.74	106	95	106	5.65
XXII	4	Controls	146–213 (av. 169)	227	202	1.925	0.081	4.23				

we see that the average for 12 controls (90 to 160 days old) was 4.26 per cent while only two test animals fell as low as this (one of these was of abnormally light body and brain), and the averages were 4.41 per cent and 4.74 per cent for four and one-half months and eight months respectively.

5. *Summary*

1. The results of the present experiments agree with those of previous investigators in that they show no marked effect of exercise either upon body length or body weight in the albino rat.

2. The female albino becomes very active earlier than does the male but the activity of the male later increases to such an extent that the total activity for the two sexes for long periods is probably about equal.

3. These experiments suggest that there is an increase in fertility correlated with increase in the size of the reproductive organs.

4. The brain weight is slightly increased by exercise.

5. The weight of the olfactory bulbs of albino rats exercised in revolving-cages for periods of from thirty to one hundred days, is considerably increased. The bulbs of such rats form from 4.41 to 4.74 per cent of the total brain weight as compared with 4.20 to 4.32 per cent in rats reared under normal colony conditions. These bulbs show an increase of 5 to 11 per cent over and above the increase in weight manifested by the entire brain.

IV. CONCLUSIONS

From the preceding observations we may conclude that we are able to modify the olfactory bulbs of the rat by changing the conditions under which it lives and to modify them to a considerably greater degree than we can change the rest of the brain. In cases of stunting, the bulbs tend to overcome the effect, to a certain extent, as time goes on. With exercise the effect seems to increase with age. Yet the bulbs respond more markedly to the stunting effect of defective feeding or sickness than to the stimulating effect of exercise.

A histological study of these modified bulbs will be presented in the second part of this paper.

V. LITERATURE CITED

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PART II. ON THE NUMBER OF NERVE CELLS IN LARGE AND SMALL OLFACTORY BULBS

CONTENTS

I. INTRODUCTION

The foregoing studies have shown that it is possible to change the relative weight of the olfactory bulbs in the albino rat (Holt, '17). This relative weight is decreased by a defective diet and increased by exercise. Such being the case, it seemed very desirable to make a histological comparison of the bulbs which had been stunted by a defective diet or enlarged by exercise, with those of rats reared under normal colony conditions. For this purpose it was of course essential to find a method of fixation and treatment which would give uniform results. Fixation in Ohlmacher's solution as recommended by King ('10) for the study of cortex cells, gave very satisfactory results, but her statement that "various individuals react differently although subjected to the same course of treatment," and her tables (loc. cit., p. 231) showing a variation in shrinkage ranging from 2 to 18 per cent in brains so fixed, suggested that it would be best to examine this method a little more in detail. Unless uniform results could be obtained by it, this method would, of course, be un-

suited to comparative study of the size of bulb elements. Accordingly, the method was further tested, and at the same time an examination was made of the effect of Müller's fluid and of Orth's Formol-Müller solution upon the various parts of the brain.

A long series of experiments demonstrated quite conclusively the following points which have an important bearing upon the present investigation.

1. Of the three fixing fluids tested, Ohlmacher's solution causes the least change in weight in brain tissue.

2. Orth's solution (cold) causes a slight increase in weight.

3. Müller's solution causes a very considerable increase in the weight of brain tissue as has already been noted (Donaldson, '94).

4. Olfactory bulbs, fixed in Ohlmacher's solution, reach a state of equilibrium at the end of twenty-four hours; fixed in Formol-Müller, they reach this state at about the end of one week; fixed in Müller's solution alone, changes continue from six weeks to two months.

5. There seems to be no appreciable individual variation in the reactions of albino rat brains of like age to Ohlmacher's solution, to Müller's fluid, or to the Formol-Müller solution. The results obtained by Dr. King are due apparently to the fact that the brains which were weighed in her experiments had been fixed for varying short lengths of time and the initial changes in weight were so rapid that there appeared to be a considerable difference in the way the various brains reacted to the fixative, when in reality, had all the brains been fixed for exactly the same length of time, no such large disagreement would have been found.

II. THE PROBLEM OF SIZE DIFFERENCES

Although under normal conditions, there is a good deal of variation in the size of the olfactory bulb of the albino rat, we have found that it is possible, experimentally, to increase this range of variation to a very considerable degree. The question next arises as to the structural cause of the difference

in size. Is it one of size of elements or of their number? Have we more cells and fibers in the heavier bulb or are the cells and fibers merely of a larger size? The present paper deals only with the question of cells. The fibers have yet to be examined.

III. TECHNIQUE AND METHODS OF STUDY

Since experiments on the effect upon the rat brain, of Ohlmacher's, Müller's, and the Formol-Müller solutions have demonstrated that for brains of like ages there is a definite, practically unvaried, swelling or shrinking reaction for any given fluid, the brains of the rats used in the defective diet and exercise experiments were fixed in these several solutions for histological comparison. For the present cell study the method adopted was that recommended by King ('10) for study of the cortex, namely fixation in Ohlmacher's solution for twenty-four hours, followed by one hour in 85 per cent alcohol, three to four days in iodized 70 per cent alcohol, double embedding in celloidin and paraffin, and staining in carbol-thionin and eosin. However, after the first trials, this method was varied in the matter of embedding. For such small objects as the olfactory bulbs, paraffin proved more satisfactory when used alone. Sections were cut 8μ thick and mounted serially. A rather deep thionin stain gave the best results for cell enumeration.

1. Preparation of sections

At first, some bulbs were cut sagittally and the largest sections compared. In the study of these sections the number of cells in the gray layer of the different bulbs was found to be so nearly identical that it was decided to attempt a thorough study of cell number.

In dissecting a rat brain into its parts, the bulbs are cut from the brain in such a way as to leave an appreciable portion of the bulb attached to the cerebrum. The method followed was to place the brain, ventral side down, on a flat surface and with a knife held in a plane perpendicular to the table, to sever the bulb at the point where it disappears beneath the cerebrum (plate 1).

This is the part of the bulb which is weighed, and since all bulbs are removed in the same way, it has been assumed that we have corresponding portions for comparison. Because the recorded weights represented only this portion of the bulbs, it seemed advisable at first to compare the cell elements of these parts only. Accordingly cross sections of the bulbs were made but unfortunately, as appeared later, most of the test series were not complete for the portion of the gray substance beneath the hemispheres. The method of meeting this difficulty will be described later.

2. Methods of study

The study of sections was made largely with the aid of the Edinger projection apparatus. Cell counts were made by projecting the sections onto white wrapping paper, outlining the area, and punching the image of each cell nucleus with a tallying register fitted with a sharp prong in place of the usual blunt register arm (Hardesty '99). The hole punched by this prong insured against counting the same cell twice. It also left a permanent record of any particular region which could be reexamined later. In some cases the count for each section was recorded; in others, the whole number of sections to be counted were registered consecutively and no record made until the end. Occasionally a section was recounted—to serve as a check on the work.

IV. GENERAL DIFFERENCES IN SIZE

Most of the comparisons of size and the determinations of cell number have been made on bulbs stunted by a defective diet, and on their respective controls. Only two bulbs of the exercise series have yet been examined.

The general differences in size between young and mature, stunted and normal olfactory bulbs are very well illustrated by the sections shown in Plates 1, 2, and 3. Figure 1 of plate 1 is a camera drawing of a median sagittal section through the bulb of a rat stunted by feeding for thirty-one days on a corn diet. The body length was 138 mm.; brain weight, 1.547 grams and the weight of the corresponding bulb which was removed and

weighed, was 0.020 gram—the approximate weight then of the bulb shown in the drawing. Figure 2, plate 1, is a median sagittal section through the control bulb. The rat from which this was taken was 166 mm. long, with brain weight of 1.698 grams, and the weight of the corresponding bulb was 0.032 gram. Like the bulbs of very young rats, the gray layer of the stunted bulbs extends somewhat further, in proportion, beneath the cerebrum than in the case of normal older individuals.

When the stunted bulb is compared with its control, there appears to be a rather uniform size difference involving all parts of the bulb. It is hard to compare the outer fiber layers owing to the difficulty in removing the bulbs perfectly from the skull. The anterior end of the fiber layer is very likely to be entirely torn away and sometimes the ventral side also suffers. However, it is plain that the glomeruli of the larger bulb are much larger and more open; the granular cells are not so closely packed together; and the gray layer is usually broader in the larger bulb and the inner granular area considerably more extensive. These differences between the peripheral portions are illustrated by the more highly magnified mid-dorsal areas *S* and *S* of figures 1 and 2, shown in figures 3 and 4 of plate 2.

Plate 3 shows three cross sections; through Q_5, a thirty-day control bulb (fig. 5); M_4, a sixty-two-day stunted bulb (fig. 6); and M_5, the sixty-two-day normal control (fig. 7), for M_4. These sections were made through the region where the bulbs are usually cut from the brain. The figures illustrate another typical difference. The normal bulb (figs. 5 and 7), as it grows, elongates more rapidly than it increases in thickness and it tends to grow faster dorso-ventrally rather than laterally. In these figures, the outer fiber layer is probably complete at the sides. Ventrally it has doubtless been torn away to some extent in all three bulbs. The difference in size of the glomeruli is well shown here, but while there is a greater area of gray matter in figure 7 than in the other two, the gray layer seems narrower than in M_4 (fig. 6). The companion bulb of Q_5, (fig. 5) weighed 0.024 gram, that of M_4 (fig. 6), 0.025 gram while M_5 weighed 0.037 gram (fig. 7). The portion of Q_5 anterior to the section

illustrated, was about 1350μ long, while M_4 had 1500μ anterior to the section, and M_5, 2000μ. The differences in size are confined to no one region but are distributed somewhat proportionally through the different layers.

V. COMPARISON OF CELLS OF GRAY LAYER

1. Size and number of small cells in molecular layer

It has been the general impression that, within certain limits, the size and weight of the brain are indices to its functional capacity. In the phylogenetic series, of course, it is, with one or two exceptions, true that increase in intelligence is accompanied by increase in the relative size of the brain. So within any given species of mammals, it has been assumed that the more efficient brain is the larger and heavier.

The question as to whether, within such a group, increase in size of the brain means an increase in the number of nerve elements or in the size of the elements themselves, becomes an important one. For an increase in the number of elements should give increased functional possibilities. So, if we find in comparing large and small brains or divisions of brains from closely related animals, that the larger structure contains a greater number of cells and fibers, then we have reason to expect from the larger and more complex structure the greater degree of efficiency.

If, on the other hand, the number of elements is found to be uniform for the part under consideration, then we must conclude that the large and the small brains have potentially the same ability to function, save as their efficiency may be affected by the size or degree of development of the individual elements.

The small cells of the molecular layer (*mo*, fig. 2) show more cytoplasm; or perhaps we may say that it is possible to distinguish more cells with cytoplasm in the molecular layer of large bulbs than of small ones. For example, the section of F_1 shown in figure 1 shows 68 cells between mitral layer and glomeruli, in which cytoplasm may be distinguished, while the control, F_5, shows 158 such cells. Corresponding sections through M_1,

a thirty-day control, and C_3, a sixty-day underfed bulb, show 108 and 103 cells with cytoplasm.

Although a difference in cell size appeared, there seemed to be little difference in numbers of cell elements in the gray layer. Although it is not always possible to distinguish between the nuclei of very small cells and possible cross sections of fibers under the conditions used for counting—yet the error due to this difficulty is probably negligible. A preliminary count was made of all elements, having the appearance of nuclei in the largest sections of the bulbs F_1, C_3, F_5, F_6, and M_1, with the following results.

TABLE I

INITIAL CONTROL				TEST				FINAL CONTROL			
Bulb	Age	Bulb weight	Number cells	Bulb	Age	Bulb weight	Number cells	Bulb	Age	Bulb weight	Number cells
	days	*grams*			*days*	*grams*			*days*	*grams*	
M_1..	30	0 029	2172	F_1	62	0 020	2569	F_5	61	0 032	2569
				C_3	59	0 021	2604	F_6	61	0 033	2693

These counts for the test and final control bulbs suggested so strongly that the number of cells is the same for bulbs of different sizes that attention was turned entirely to the investigation of this point. At first longitudinal sections were used, but these were soon abandoned for two reasons. First, it seemed desirable to be able to count the cells of just that portion of the bulbs corresponding to the part weighed; and second, the longitudinal sections presented so many irregularities that it was necessary to count many more sections to approximate the true average than in the case of the cross sections. Counts were made of all elements in the gray layer outside the mitral layer between the tip of the bulb and the point at the proximal end where the gray layer is first interrupted on the dorsal aspect of the bulb (see figs. 5, 6, 7). These counts consumed a vast amount of time and when completed seemed to disagree with the observations already made upon the longitudinal sections (table 2).

A first glance at the table would indicate that the small bulb has fewer cells and would suggest that this difference in cell

bulb. The number for X_6, whose weight was 0.040 gram, was 73,950. To see whether there were any virtue in making so thorough a count of cross sections, the total number was computed from a recount of every 10th section, excepting at the most anterior end where every cell was counted in every section, until the sections showed a single layer of mitral cells. By this method the number obtained for X_1 was 64,775 cells, making a difference of only 0.4 per cent. For X_6 the count was 73,324, which was 0.8 per cent smaller than the more exact count obtained by counting half the sections. These differences were so small as to make the more exhaustive count seem unnecessary. X_6 gave an almost complete series through the entire gray layer so the count was completed, giving for the entire bulb 80,114 cells. The count of the mitral cells in X_1 could not be completed as the series had been cut, unfortunately, with the idea of comparing only the parts of the bulbs whose weights we knew, and which, therefore, extended back but a short distance under the cerebrum. The cells of these few sections were, however, counted, giving a total of 71,914.

The number of mitral cells in G_6 was computed from absolute counts of anterior and posterior ends of the series and by counting every tenth section through the rest of the series. M_4, M_5 and Q_5 ran so evenly that here in the middle portion of each series, only every twentieth section was counted; on either side of this portion, every tenth section, and all cells of all sections at either end.

With the sagittal sections, the task was more difficult and the results, I believe, less reliable for this reason: toward the sides of the bulbs, especially the median side, the sagittal series may give tangential sections of the mitral layer so that a single section may yield a count of 1500 cells whereas a section two or three removed on either side might have but 300 or so mitral cells. It can be easily seen that if the section to be counted, happened to fall in such a region, or entirely skipped such a region, the count would be considerably modified. Some of the bulbs gave no trouble of this kind while others were hard to count for this reason. G_3 was an interesting example of the way this

may work out. A count was first made of all cells at either end of the series and those in every tenth section through the middle portion. The result when computed was 95,993 mitral cells. Then the middle section of every ten was counted with a total result of 83,974 cells. Two other series were attempted but abandoned as the bulbs were so irregular that an accurate count would have required the enumeration of the cells of at least every alternate section. The other bulbs, except C_3 for which every fifth section was counted, were fairly regular so that the mitral layer offered no such complications. For these, the method of counting all cells at either end of the series and those of every tenth section through the median portion was followed. The sequence of counts was varied with each bulb, and the records kept in various ways and not infrequent recounts made. The recounts were surprisingly close to the original, for, as has been stated, it is not always easy to decide whether or not a cell should be counted, and in focusing as one counts, a granule lying below or above a portion of a mitral cell sometimes looks very like a nucleus, but the error due to this cause is probably too small to be considered.

Details of counts of the different bulbs, arranged in the order followed in table 3.

Bulb X_1, thirty day, initial control. Cross sections. Mitral cells counted in every section of anterior end back to the first section in which the mitral cells appeared in a single layer. From this point, counts were made for every tenth section back to the cerebrum. By computation, the total number of mitral cells was 64,775. By a recount in which the mitral cells of every other section were enumerated the computed number was 64,470 making a difference of only 0.4 per cent in the two counts. The series of sections for the region beneath the cerebrum was incomplete, the posterior portion not having been preserved. A count was made, however, of the sections which were present. This number added to the number already counted by the second method, brought the total up to 71,914 cells.

Bulb E_1, Test, defective diet series. Sixty-two days. Sagittal sections. Mitral cells counted in all sections at either end of the series and for every tenth section between.

Bulb C_2 Test, defective diet series. Fifty-nine days. Sagittal sections. Counts made as in E_1.

Bulb Q_2, thirty day, initial control. Defective diet series. Cross sections. Mitral cells counted in all sections at both ends of the series.

the lateral ventricles until the end of the second year. Hatai observed an increase in number of cells in the spinal ganglia, corresponding to increase in age but this increase was attributed in part, at least, to failure to count all the ganglion cells in very small animals. Ranson ('06) in a study of the second cervical nerve found no correlation between the number of cells and the number of myelinated fibers, neither did he find the number of cells to vary with the age of the rat.

The results of the present investigation of the number of cells in the olfactory bulb help to confirm the impression that the number of cells in the central nervous system becomes fixed at an early age so that after the first three or four weeks at least, there is no material change in the cell number.

This study also gives us reason to believe that the number of small and of mitral cells in the gray layer of the olfactory bulb is very nearly the same for all individuals with especially close agreement between individuals of the same litter. It seems fairly evident that while external conditions may modify to a considerable extent the size of the brain of the albino rat and especially the size of the olfactory bulbs, the only effect is upon the relative development of the individual cells. The number of cells remains the same. The fibers have yet to be examined.

It is important to bear in mind in a determination of this sort—e.g., the number of mitral cells—that a fixed number, in the physical sense, is not to be expected, for all organisms are normally variable in all of their parts, variability being an essential character for living things; so the number which is obtained gives a mean value which we take to be characteristic for the species under the present conditions, but around which equally characteristic variations also occur.

VI. CONCLUSIONS

1. For bulbs of different ages and sizes, the regions anterior to the cerebrum, which are commonly considered the bulbs, are not strictly homologous, since, in the brains of young or stunted rats, a larger proportion of the bulb lies beneath the cerebrum than in the case of the better developed brains.

2. All layers of the olfactory bulb are about equally concerned in the increase in size or in the arrest of development of the bulb.

3. The small cells of the molecular layer show a larger amount of cytoplasm in large bulbs than in small ones.

4. The number of small cells in the molecular layer, apparently, is not correlated either with age of the rat or size of the bulb. The entire computed number for a small, medium, and large bulb was found to be approximately 1,000,000 cells ± 2 per cent.

5. The mitral cells of small bulbs are smaller, on the average, than those of large bulbs.

6. Within the limits here taken the number of mitral cells is not affected by the age or the size of the bulb.

7. There seems to be some variation between litters in the number of the mitral cells. The average number of mitral cells for 13 bulbs was 76,750, the lowest number being 70,625, and the highest 83,974. The standard deviation σ is 4564 and the probable error of the mean ± 855.

8. When members of the same litter are compared, bulbs stunted by a defective diet or enlarged by exercise show practically the same number of mitral cells as do their controls. The mean difference is −1.8 per cent for the tests.

9. The olfactory bulb size is correlated with cell size and not with cell number.

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ABBREVIATIONS

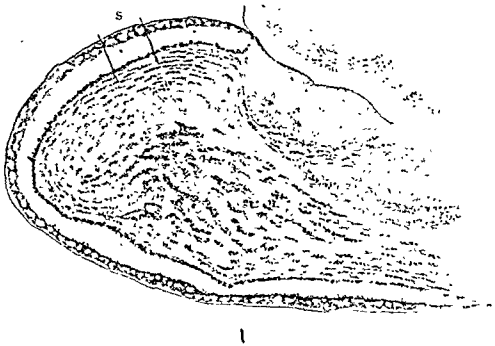
s, areas in figures 1 and 2 enlarged in figures 3 and 4.
fi, outer fiber layer
gl, glomeruli
mo, molecular layer
mi, mitral layer
g, granular layer
f, inner fiber layer
c, cerebrum

PLATE 1

EXPLANATION OF FIGURES

Median longitudinal section through olfactory bulb of F_1, section 3 1/6. F_1, underfed 31 days. Final brain weight, 1.5470 grams, bulb weight, 0.0203 gram. Defective diet. Magnified 24 diameters.

Median longitudinal section through olfactory bulb of F_2, section 5 5/4. F_2, control for F_1. Brain weight, 1.6984 grams, bulb weight, 0.0315 gram. Magnified 24 diameters.



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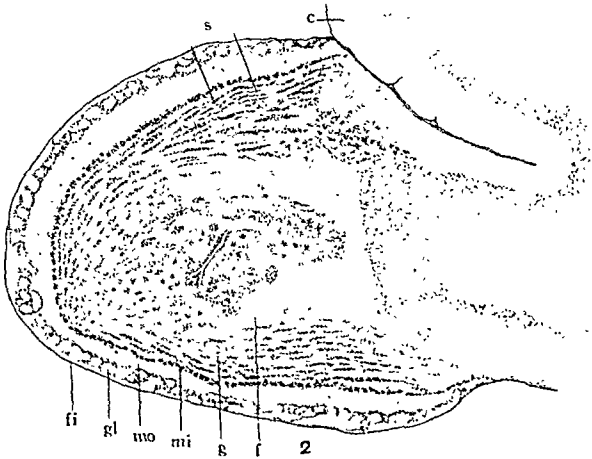
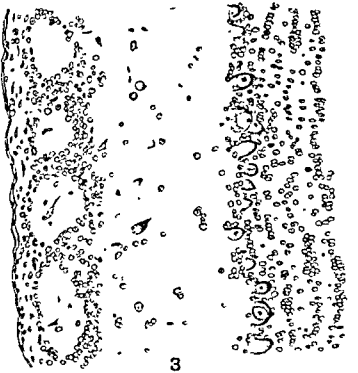


PLATE 2

EXPLANATION OF FIGURES

Portion of section of F_1; area S, in figure 1. Magnified 172 diameters.
Portion of section of F_5; area S, in figure 2. Magnified 172 diameters.



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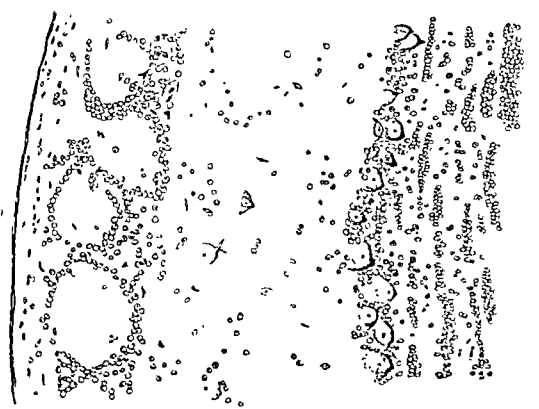

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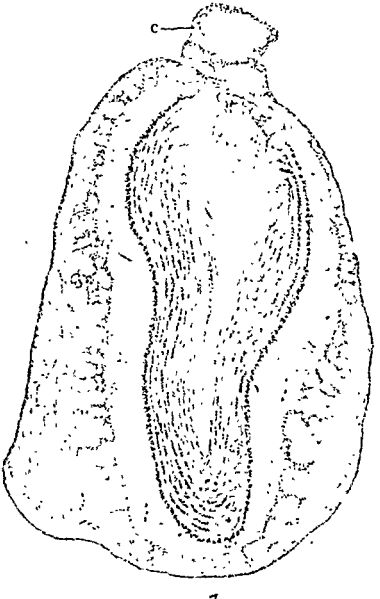
PLATE 4

EXPLANATION OF FIGURES

Cross section of M_5, section 3 6/12, cut same as figure 5 above. M_5, control for M_4. Brain weight, 1.7110 grams; bulb weight, 0.0374 gram. Magnified 30 diameters.



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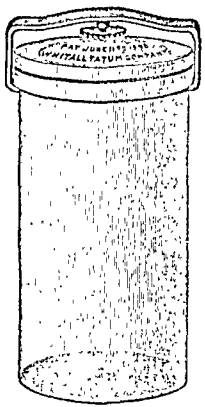

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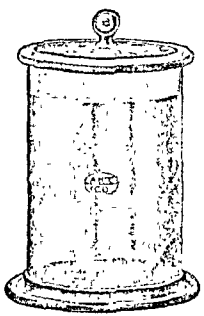

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No. 4. The Development of the Lymphatic System in Fishes, with Especial Reference to its Development in the Trout, by C. F. W. McClure, Department of Comparative Anatomy, Princeton University. 140 pages, 41 figures, 11 of which are in colors. Sent post paid to any country for $2 50. 1915.

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No. 6. The Rat, compiled and edited by Henry H. Donaldson Reference tables and data for the Albino Rat (Mus Norvegicus Albinus), and the Norway Rat (Mus Norvegicus), 280 pages. Sent post paid to any country for $3 00. 1915

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FURTHER CONTRIBUTIONS ON NEUROBIOTAXIS

IX. AN ATTEMPT TO COMPARE THE PHENOMENA OF NEUROBIOTAXIS WITH OTHER PHENOMENA OF TAXIS AND TROPISM. THE DYNAMIC POLARIZATION OF THE NEURONE

C. U. ARIËNS KAPPERS

From The Central Institute for Brain Research, Amsterdam

SIX FIGURES

CONTENTS

NEUROBIOTAXIS AND ITS SELECTIVE CHARACTER

In various articles,[1] first in 1907, I have published observations concerning the shifting of nerve cells in the central nervous system, which could be shown by the different places that

[1] The principal points are mentioned in:

Die phylogenetische Verlagerungen der motorischen Oblongata-Kerne, ihre Ursache und ihre Bedeutung. Neurologisches Centralblatt, 1907, and Rapport du Congrès international de Psychiatrie et de Neurologie, Amsterdam, 1907.

Weitere Mitteilungen über die Verlagerungen der motorischen Oblongata-Kerne: der Bau des autonomen Systems. Folia Neurobiologica, Bd. 1, 1908.

Specially in: Weitere Mitteilungen über Neurobiotaxis. Die Selektivität der

THE JOURNAL OF COMPARATIVE NEUROLOGY, VOL. 27, NO. 3
APRIL, 1917

the motor nuclei of the oblongata exhibit in the series of vertebrates.

Ontogenetically the same phenomenon could be stated. Since it was most evident that the shifting of these central groups took place in the direction of the point whence the majority of stimuli proceeded to their cells, we apparently had to do with a phenomenon of taxis or tropism, which I called Neurobiotaxis, because it occurs in the nervous system during life (in its phylogenetic and ontogenetic development) and I did not know where

Zellen-Wanderung. Die Bedeutung synchronischer Reizverwandtschaft, etc. Folia Neurobiologica, Bd. 1, 1908.

Über die Bildung von Faserverbindungen auf Grund von simultanen und successiven Reizen. Bericht des III. Kongresses für experimentelle Psychologie in Frankfort am Main, 1908.

Further anatomical details are found in:

Weitere Mitteilungen über Neurobiotaxis, II. Die phylogenetische Entwicklung des horizontalen Schenkels des Facialiswurzelknies. Folia Neurobiologica, Bd. 2, 1908.

Weitere Mitteilungen über Neurobiotaxis, III. Über den Einfluss der Neurone der Geschmackskerne auf den motorischen Facialis und Glossopharyngeuskern und ihr Verhalten zur Radix descendens Nervi quinti. Folia Neurobiologica, Bd. 3, 1909.

Weitere Mitteilungen über Neurobiotaxis, IV. The migrations of the abducens nucleus and the concomitating changes of its root-fibers. Psychiatrische en Neurologische Bladen, Amsterdam, 1910.

The migrations of the motor cells of the bulbar Trigeminus, Abducens and Facialis in the series of vertebrates and the differences in the course of their root-fibers counted as Mitteilung V.). Verhandelingen der Kon. Akad. v. Wetenschappen, Amsterdam. Tweede Sectie, Deel 16, Nr. 4, 1910.

Weitere Mitteilungen über Neurobiotaxis, VI. The migrations of the motor root-cells of the vagus group and the phylogenetic differentiation of the hypoglossus nucleus from the spino-occipital system. Psychiatrische en Neurologische Bladen, Amsterdam, 1911.

Weitere Mitteilungen über Neurobiotaxis, VII. Die phylogenetische Entwicklung der motorischen Wurzelkerne in Oblongata und Mittelhirn. Folia Neurobiologica, Bd. 6, Sommergangzungsheft, 1912.

Weitere Mitteilungen über Neurobiotaxis, VIII. Über den motorischen Facialis- und Glossopharyngeus Wurzel bei niederen Vertebraten. Folia Neurobiologica, Bd. 9, 1912.

The structure of the autonomic nervous system compared with its functional activity. Journal of Physiology (England), vol. 37, 1908, p. 139.

Phenomena of neurobiotaxis in the central nervous system. Section Anatomy and Embryology, of the XVIIth International Congress of Medicine, London, 1913.

to classify it under the phenomena of galvanotaxis, chemotaxis or other processes of taxis or tropism known at that time.

This phenomenon of shifting is clearly shown by figures 1 and 2, where the dorsal position of the abducens nucleus in the shark with its huge fasciculus longitudinalis posterior (*f.l.p.*, fig. 1) strongly contrasts with the ventral position of the same nucleus in a bony fish (fig. 2), where the fasciculus longitudinalis

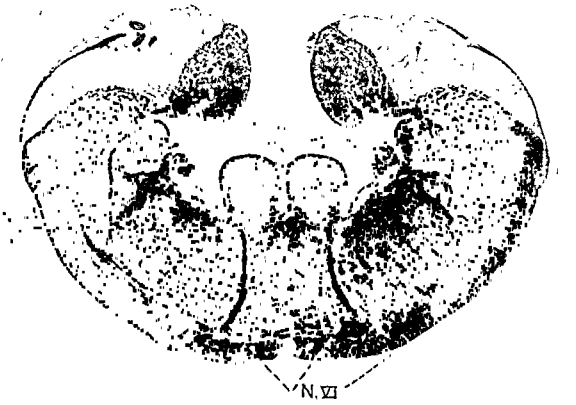

Fig. 1 Acanthias vulgaris, showing the dorsal position of the abducens nucleus. *f.l.p.*, *fasciculus longitudinalis posterior*; *Nuc.VI*, *abducens nucleus*; *N.VI*, abducens nerve; *r.VII,m.*, motor facialis root. After Van der Horst.

posterior is much smaller, but where the ventral set of central afferent tracts which influences this cell group is much more strongly developed (tr. tecto-bulbares ventrales, *tr.t.b.v.*, fig. 2).

The first way in which I formulated this law was thus: When from different places stimuli proceed to a cell, its chief dendrite grows out and its cell-body shifts in the direction whence the

majority of stimuli proceed.[2] The truth of this was soon confirmed also in other parts of the cerebrum, by Tretjakoff,[3] Herrick,[4] Bartelmez,[5] Obenchain,[6] Bok, Van der Horst[7] and others.

I observed, however, on an increase of afferent stimuli in a given center, that not all the neighboring cells approach this center, but that only certain cells proceed to that center which apparently had a certain relation to it, while other cells (even lying nearer by) did not migrate into the direction of the increased sensory field, because evidently they had nothing to do with it and did not stand in relation to it.

Further researches convinced me that the functional relation which appeared to be the condition for the approach was shown to be a correlation depending on simultaneity of function—of stimulation.

So the abducens nucleus shifts from one center of visual co-ordination fibers (the *f. l. p.*) to another set of visual co-ordination fibers (the tr. tecto-bulbaris) if the latter increase; but an increase of the tecto fibers for instance, does not have any effect upon it.

[2] I soon found that a similar observation had been already made by Strasser ('92) and by Cajal ('99). Compare: Strasser, Alte und neue Probleme der Entwicklungsgeschichtlichen Forschung auf dem Gebiete des Nervensystems. Ergebnisse der Anatomie und Entwicklungsgeschichte, Bd. 1, 1892, p. 721. Cajal, Textura del sistema nerviosa del hombre y de los vertebrados, vol. I, 1899, p. 560. See also Cajal, Algunas observacionas favorables a la teoria neurotropica. Trabajos, vol. 7, 1908, p. 63. Both, however, failed to see the correlative character in this process, and Cajal ascribes a great influence to the spongioblasts (ependyma and glia) in the secretion of attracting chemicals for the axones, in which I do not at all agree with him.

[3] Tretjakoff. Das Nervensystem von Ammocoetes, II. Das Gehirn. Archiv f. mikrosk. Anat., Bd. 75, 1909.

[4] Herrick. The morphology of the forebrain in Amphibia and Reptilia. Jour. Comp. Neur., vol. 20, 1910.

[5] Bartelmez. Mauthner's cell and the nucleus motorius tegmenti. Jour. Comp. Neur. vol. 25, 1915.

[6] Obenchain (with Herrick). Notes on the anatomy of a cyclostome brain, Ichthyomyzon concolor. Jour. Comp. Neur. vol. 23, 1913.

[7] Van der Horst. De motorische kernen en banen in de hersenen der visschen, hare taxonomische waarde en neurobiotactische beteekenis. See also Tijdschrift der Ned. Dierk. Vereen. 1917.

Then I found—though not starting my work with a psychological scope—that the anatomical relations of the dendrites and the cells in the nervous system were regulated in accordance with the law which, in psychology, is known as the law of association, in which law (in all the different forms[8] in which it may appear) the simultaneity of stimulations or residua of stimulations is the essential part.

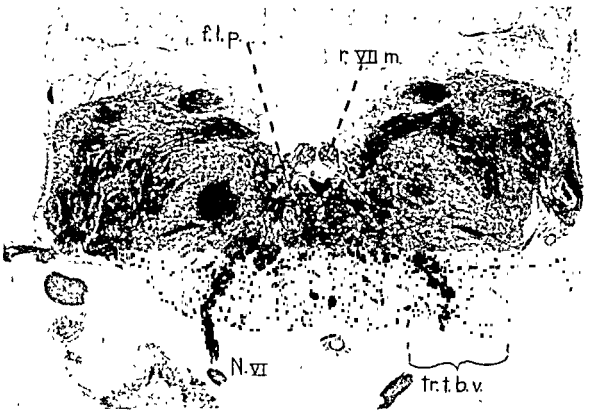

Fig. 2 Tetrodon, showing the ventral position of the abducens nucleus. *f.l.p.*, fasciculus longitudinalis posterior; *Nuc.VI*, abducens nucleus; *N.VI*, abducens nerve; *r.VII,m.*, motor facialis root; *tr.t b.v.*, tractus tecto-bulbaris ventralis. After Van der Horst

This anatomical observation, first made on motor cells, led me to study more carefully the courses of several axon-tracts, sensory tracts, as well as the so-called "central motor tracts," such as the pyramids, and it soon appeared to me that a criti-

[8] Those forms are simultaneity, successivity, similarity and contrast. In the three first named forms the presence of one stimulus, or remains of a stimulus, while the other is added, is obvious. The association by contrast is also due in the first place to simultaneity of impression since the simultaneous or successive contrast makes us discriminate things: black and white, father and mother, etc.

cal study of their relation showed most clearly that the same law of neurobiotaxis, the simultaneous relationship in their stimulative function, had been the cause of their final arrangement.[9] So I was able to formulate the phenomena of neurobiotaxis in the following words:

I. If in the nervous system several stimulation-charges occur, the growth of the chief dendrite, and eventually the displace-

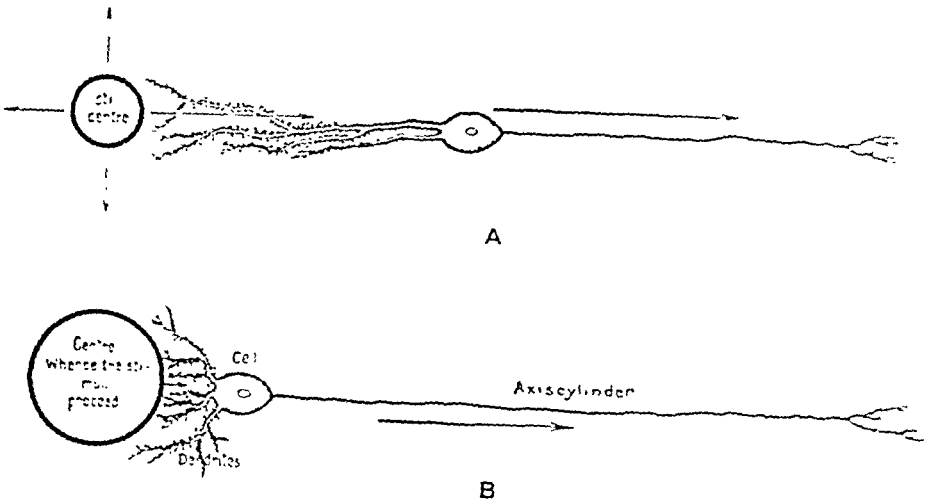

Fig. 3. Showing that, while the axis-cylinder runs with the direction of the nervous current, the dendritic outgrowth and the final shifting of the cell body occur against the nervous current. *A*, giant dendrites grown out towards the center of stimulation. *B*, the cell body (perikaryon) has shifted toward the center of stimulation; the axis-cylinder is consequently elongated.

ment of the cell-body itself, takes place in the direction, whence the majority of stimuli proceed to the cell.

II. Only between correlated centers does this outgrowth or shifting take place.

III. The growth of the axis-cylinder (i.e., its final connection) is not primarily regulated by motor centers,[10] but also here synchronic or successive stimulation (correlation) acts a part.[11]

[9] Folia Neurobiologica, Bd. I, 1908.

[10] Not by some undefined transcendental willing (teleologically).

[11] That is, it is defined by correlation.

While, however, it was evident that the approach of the dendrites and nerve cells to a territory (fig. 3) took place towards the center of the stimulation (as a stimulopetal or centripetal tropism), that is, against the nervous current of stimulation proceeding from this center, the problem became much more difficult to explain how the connection between correlated centers was effected by the axis-cylinder, since it was obvious that the axis-cylinder does not grow towards the stimulation (stimulopetal) to meet it, but moves in the same direction as the stimulus-irradiation (stimulo-fugal or centri-fugal).

BOK'S RESEARCHES: THE STIMULOGENOUS FORMATION OF THE AXON

That the axis-cylinder really grows with the current and that the irradiation of this current plays an important part in its growth has been proved and very carefully examined in this Institute by S. T. Bok, who got highly important results.

Bok[12] found that when an axis-cylinder or a bundle of amyelinated nerve-fibers grows out and passes nerve cells on its way, these nerve cells can be activated to send out an axis-cylinder of themselves in a region perpendicular to the activating axon or bundle (fig. 4).

This fact was found with the fasciculus longitudinalis posterior in such a form as left no doubt, since it appeared that the motor nuclei which undergo the influence of this bundle were only activated according to the degree in which the fasciculus longitudinalis posterior had grown out. So the axons of the trigeminal[13] cells first grow out, then follow the axons of the facialis cells, then those of the glossopharyngeus and vagus.

The same was seen in the activation of the oculomotorius, abducens and hypoglossus nuclei which are activated by another influence of the same character.

[12] Bok. Die Entwicklung der Hirnnerven und ihrer Zentralen Bahnen. Dei Stimulogene Fibrillation. Folia Neurobiologica. Bd. 9, 1915. See also Bok, Stimulogeneous Fibrillation. The cause of the structure in the nervous system. Psych. en Neurologische Bladen, Amsterdam, 1915.

[13] Concerning the Trochlearis. See the first-named original.

Bok, considering the fact that the formation of the axis-cylinders in those cells took place under the influence of the current irradiating from the primary activating axis-cylinder, called this stimulogenous fibrillation, following the direction of that current in contrast to the outgrowth of the dendrites and

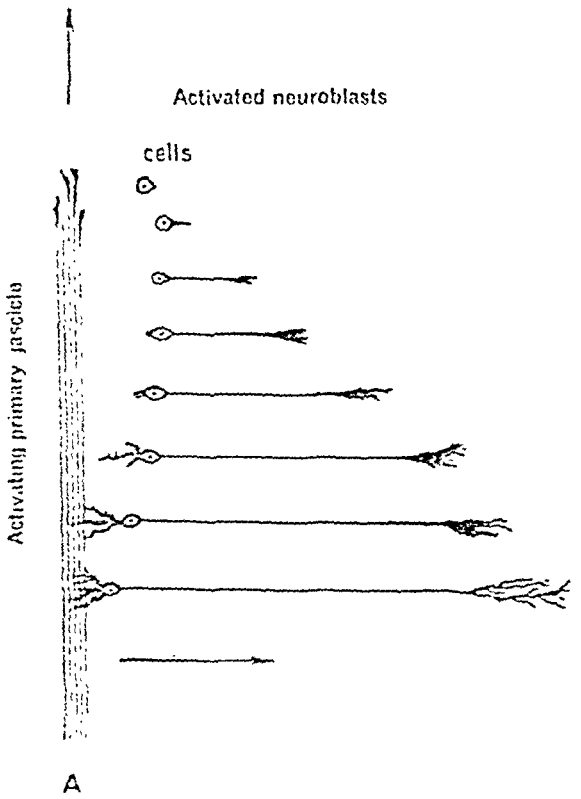

Fig. 4. The activation of adjacent neuroblasts by an amyelinated (growing) fascicle. The vertical arrow indicates the direction of growth of the activating bundle and the direction of its nerve current, which starts at A. The horizontal arrow indicates the course of the irradiating influence (current) perpendicularly to the activating bundle. Notice that the proximal cells are sooner activated (and have moved further) than the more distant ones. After Bok.

the shifting of the cell body,[14] both of which also only occur later and which move towards the center, i.e., against the current of the stimulus that proceeds to them.

This observation and Bok's interpretation of it are very important, and no doubt correct. It is evident, however, that the final end-point of the growing axis-cylinder can not be determined by this process alone, as was also realised by Bok, who came to the conclusion that the final connection was determined by the principal law of neurobiotaxis, viz., by the stimulative (simultaneous) correlation of the growing axis-cylinder and its end-point, i.e., the cell or dendrites with which it is going to be connected.

Bok thought that this could be effected by the fact that if two centers are in simultaneous stimulation the ideal line between the two is the path where the plasmodesms undergo the greatest influence of this relation. He called this the principle of the 'doppelte Bahnung,' and thought that Einstein's (physical) law of attraction between synchronic energies also had some influence on it.

It seems to me, however, that the principle of 'doppelte Bahnung,' as laid down in this theory, can not explain from which of two simultaneously stimulated cells the axis-cylinders grow out, and that even the adaptation of the protoplasm to the formation of the axis-cylinder, eventually a fibrillation of the neurodesms, then might begin in the middle between two cells which, as we know, it never does. Moreover, the expression "adaptation of protoplasm to its biological function" is too general an expression to explain anything.

It has appeared to me that the literature of recent years concerning the microchemistry of the neurones and the phenomena of tropism and taxis known and experimentally examined in other organisms, together with Bok's discovery, concerning the

[14] If the normal *stimulation* of the cell body is of little importance or eventually absent, the cell may also shift in the same direction in which the axon grows out. (See my paper on the autonomic nervous system. Journal of Physiology, 1908, vol. 37, p. 139.)

stimulogenous outgrowth of the axis-cylinders from the activated cell by and with the irradiating current from a primary or activating axis-cylinder in its neighborhood, gives us a key of exceptional importance to comprehend the phenomena of neurobiotaxes in general, and the contrasting behavior in outgrowth direction between dendrites and axons and allows us to consider, perhaps to explain, *how it is possible that a unit such as the neurone is may exhibit two opposite directions of growth.*

EXPERIMENTS CONCERNING PHENOMENA OF TROPISM AND TAXIS IN PLANTS AND ANIMALS KATAPHORETIC PHENOMENA

It is evident that, in any attempt to explain the neurobiotactic phenomena, these must be compared with other phenomena which are better adapted to experimental investigation. As such we may mention the galvano-tropic phenomena in the growth of plant-roots and the orientation of animals in the constant current, about which we have obtained many data during the last decennia.

As is known, the phenomenon of galvanotropy in plant-roots was discovered in Hermann's laboratory by Müller-Hettlingen,[15] who found that, if the sprouting seed of the bean (Vicia faba) be exposed to a constant current, the tips of the root turn and grow towards the negative pole (kathode).

An analogy[16] of this galvano-tropic phenomenon is found in the galvano-tactic phenomenon described by Bancroft,[17] viz., that the tentacles and the manubrium of a medusa, Polyorchis, during the transmission of a constant current turn towards the kathode.

In the experiments with the latter this peculiar phenomenon was observed, viz., that with a long-continued current the side turned to the anode extended, becoming thinner and weaker; this last phenomenon being a symptom of decay, according to this author (vide infra).

[15] Müller-Hettlingen. Ueber galvanische Erscheinungen an Keimenden Samen. Pflüger's Archiv, Bd. 31, 1883, p. 192.

[16] Not a homology, probably.

[17] Jour. Exp. Zoöl., vol. 1, 1904, p. 289.

As third example of galvano-taxis the phenomenon discovered by Verworn[18] in one-celled creatures must be mentioned here, viz., that these (ameba, for instance) on the transmission of a constant current through the surrounding medium, send out enlargements and finally shift in the direction of the kathode.

Verworn at first held the opinion that there were also Protozoa which shift under normal circumstances to the anode, and he therefore made a distinction between kathodic and anodic galvano-taxis. Later investigations revealed that the anodic galvano-taxis must be considered as being something different from the kathodic, and that the direction and shifting of the bodies observable in Protozoa under normal circumstances is invariably a *kathodic galvano-taxis.*

Also Boruttau (personal communication) holds the opinion that every real galvano-tropism is a kathodic stimulation phenomenon. This usual kathodic galvano-tropism can be brought into correlation with Pflüger's law, as has been pointed out by Loeb and Maxwell.[19]

As far as the anodic tropism is concerned, Loeb and Budgett[20] and after them Cohn and Barratt[21] found that when a protozoan, Paramecium, in pure water or in a weak solution of common

[18] Verworn Die polare Erregung durch den galvanischen Strom. Pflüger's Archiv, Bd. 45, 1889. Verworn. Die polare Erregung der Protisten durch den galvanischen Strom (Fortsetzung). Pflüger's Archiv, Bd. 46, 1890. Verworn Untersuchungen über die polare Erregung der lebendigen Substanz 3te Mitteilung. Pflüger's Archiv, Bd. 62, 1896, S. 415. Verworn Die polare Erregung der lebendigen Substanz durch den Constanten Strom. 4te Mitteilung Pflüger's Archiv, Bd. 65, 1897.

[19] J. Loeb und S. S. Maxwell. Zur Theorie des Galvano-tropismus Pflüger's Archiv, Bd. 63, 1896.

See also J. Loeb und Walter Gerry. Zur Theorie des Galvano-tropismus, II. Versuche an Wirbelthieren. Pflüger's Archiv, Bd. 65, 1897, S. 41. J. Loeb. Zur Theorie des Galvano-tropismus, III. Ueber die polare Erregung der Hartdrusen von Amblystoma durch den Constanten Strom Pflüger's Archiv, Bd. 65, 1897, S. 308.

[20] Loeb und Budgett. Zur Theorie des Galvano-tropismus. IV. Mitteilung über die Ausscheidung electropositiver Ionen an der ausseren Anoden flache protoplasmatischer Gebilde als Ursache der Abweichungen vom Pflüger'schen Erregungsgesetz. Pflüger's Archiv, Bd. 65, 1897, S. 532.

[21] Cohn und Barratt. Ueber Galvano-taxis von Standpunkt der physiologischen Chemie. Zeitsch. f. allgemeine Phyziologie. Bd 5, 7, 1905.

with a surplus of positive ions, will on the transmission of the constant current pass to the kathode.

If we accept this theory as correct, we shall have to assume that in ordinary circumstances—under which the kathodic tropism or taxis predominates—also a greater charge of positive ions is present in the cell-body of the ameba, or in the protoplasm of the tentacles or root-tips, than in the surrounding extra-protoplasmatic medium. This explanation is not generally accepted, but that the condition of the extra-protoplasmic medium is of great importance has also been emphasized by Loeb and Budgett, who are equally inclined to ascribe the exceptions to Pflüger's law (the anodic migrations) to alterations in the extra-protoplasmic medium. They refer to a phenomenon which may be exhibited by that side of an ameba or paramecium that is turned to the anode, viz., the extension of the protoplasm on that side, eventually followed by liquefaction. This anodic extension, first observed by Verworn (loc. cit.), is the first thing that appears when Protozoa are exposed to the constant current and precedes the real kathodic galvano-tropism.

Loeb and Budgett (loc. cit.) have submitted it to a more detailed examination and also came to the conclusion that this process is a result of the extra-protoplasmatic medium. Their explanation of this anodic phenomenon differs from the one given by Cohn and Barratt. They are, however, equally inclined to consider this phenomenon as due primarily to changes in the extra-protoplasmatic medium[7] in contrast to the phenomena of common tropism following Pflüger's laws of irritation. It may be mentioned still that the most favorable strength of current in those experiments with ameba was only 0.4 milliampere.

Besides these galvano-tactic and galvano-tropic phenomena of living protoplasm, we know of polar phenomena in dead organic substances rendered evident by the direction in which albumen shifts when subjected to a constant current: viz., the phenome-

[7] Perhaps this mode of explanation may be also applicable to the above-mentioned reversal of the galvano-tropism of root tips and to the anodal phenomenon observed by Bancroft (vide supra).

non of kataphoresis. I refer here to the investigations of Hardy,[28] which showed that as long as an albuminous solution is alkaline the particles suspended in it shift towards the anode on the transmission of a constant current, whereas they shift towards the kathode when the solution is made slightly acid.

One is apt to look for an explanation of this also in the fact that on the boundary between a colloid particle and the surrounding fluid, a double layer in the sense of the theory of Helmholtz-Quinke is present.

If now the solution is alkaline, a transmission of ions will take place, in consequence of which the albuminous particle itself becomes negative and thus shifts to the anode on the transmission of a constant current, while in the case of an acid reaction of the surrounding fluid the contrary takes place. From the reversibility of the kataphoretic phenomenon (Hamburger)[29] the curious fact thus follows, viz., that also proteid particles have the peculiarity that their electric character is determined by the reaction of the surrounding medium.

That here too, just as in the above trópism of the root-tips, an iso-electric condition occurs is clear.

APPLICATION OF THESE EXPERIMENTS TO THE GROWTH OF THE NEUROBLAST. THE FORMATION OF THE AXON

If, with these facts before us, we consider the phenomena which appear during the formation of an axis-cylinder[30] in an activated cell (which precedes the formation of dendrites—see

[28] Hardy. On the coagulation of proteid by electricity Jour. of Physiol., vol. 24, p. 288, 1899. Proc. Roy. Soc., vol. 68, p 110, 1900

[29] Hamburger. Osmotischer Druck und Ionenlehre. Wiesbaden. Bergmann. 1904, vol. 3, p. 68.

[30] It is hardly necessary to say that the fact that isolated ganglion cells, as in Harrison's experiments, may also send out axis-cylinders proves nothing against the following text. Harrison (loc. cit., p. 833) remarks that this is a process of self-differentiation entirely independent of external conditions. This is true to a certain extent, but we must assume that before it becomes a self-differentiation its differentiation has been induced to the neuroblast in former generations by external circumstances and that its doing this by itself is based on hereditary engrammatic qualities. It is better to see a problem in things than to explain them by a word which implies a still greater problem.

fig. 4), we shall first have to mention the fact that the stimulation center with respect to the surrounding tissue is negative, forming a kathode with reference to the non-stimulated surroundings, as physiological experiments abundantly prove.

Moreover the strength of the electrolytic potential differences occurring in the nervous system in consequence of stimulation appears to be of the same category as those that are applied in artificial phenomena of galvano-taxis (see above) since it may vary from 3 millivolt to 0.8 millivolt and lower, so that the forces developed here are certainly strong enough to influence processes of formative tropism and functional taxis.

Now, it may be the same whether this stimulated center is the body surface in or under which nerve cells lie or whether we start our deductions with a primary growing axis-cylinder which on its way passes neuroblasts. This negative potential not only runs along the primary axis-cylinder (fig. 4) but also, we may assume, as long as the axis-cylinder is not provided with an insulating medullary sheath, that this negative potential stands perpendicular to the length of the activating axis-cylinder (or body surface), irradiating from it.[1]

In accordance with this perpendicular irradiation of the electrolytic influence or current, we see that the neuroblasts near the primary activating bundle send out axis-cylinders perpendicular to the activating bundle, and that similarly perpendicular collaterals may grow out from the original (activating) axis-cylinders themselves.

In both cases, in the formation of collaterals as well as in the outgrowth of the axis-cylinder of the secondary (activated) neuroblasts, the axis-cylinder substance proceeds in the direction of the perpendicular irradiation of the stimulated fiber, i.e., to the anodic pole.

[1] The irradiative stimulus of naked axons is very clearly illustrated by the position of the dendrites of Purkinje's cells perpendicular upon the parallel fibers in the molecular layer of the cerebellum and of the dendrites of the motor cells on the longitudinal (naked) axons in the spinal cord of Petromyzon. See my paper, Ueber das Rindenproblem, etc., in the Folia Neurobiologica, Bd. 8, 1914, pp. 529-570.

This first outgrowth which, in the beginning, can be complicated with an anodal katophoretic shifting of the cell body itself (fig. 4) may be entirely independent of a propagation of the nervous current itself along the newly formed short axis-cylinder. As soon, however, as this axis-cylinder is fit for nervous conduction its rate of outgrowth will be considerably increased, a much stronger negative current running in the direction of its growth to the anodal field.

Why does this anodic growth occur before the kathodic tropism of the dendrites and the cell body? I will consider this question in the light of the above-mentioned experiences.

We know that the neuroblast is embedded in an organic solution, the pericellular lymph, containing a good deal of potassium salts.

Macallum has emphasized that the amount of potassium salt external to the nerve cell is great and that a considerable condensation of this element is present on its exterior surface.

Now Verworn has shown that on the transmission of a constant current the first thing to appear is an anodal expansion of the cell body, thus showing that a change of tension may be localised, by electric influences, on the anodal pole.

We may expect that this extension, being under the influence of a considerable amount of K and Cl, derives certain chemical and tropic characteristics from it.

That this really occurs in nerve cells is proved by the chemical constituents of the axon, compared with those of the dendrites and cell body.

We know from the researches of Macdonald, Macallum, Alcock and Lynch that the axis-cylinder is distinguished from the dendrites and the cell body by a much larger quantity of potassium and chlorides[32] (which, according to Macdonald, may also contribute to its conductivity for the nervous current).

[32] MacDonald. The injury current of nerves, The key to its physical structure. Report of Thomson-Yates Laboratory, vol. 4, 1902, p. 213.

MacDonald. The structure and function of nerve-fibers. Proceedings of the Royal Society, vol. 76, B. 1905, p. 322.

Macallum. On the distribution of potassium in animal and vegetable cells Journal of Physiology, vol. 32, 1905.

Macallum. Die Methoden und Ergebnisse der Mikrochemie in der Biologis-

The large quantity of KCl then present around its colloidal substance will favor (according to the experiments of Gassner, Schellenberg, and others) the anodo-tropic character of the axis-cylinder.

The phenomenon of the formation of the axis-cylinder and its collaterals in the direction of the anodic field, may thus be so expressed that we say that the neuroblast embedded in a solution containing a good deal of potassium and of chloride exhibits, in harmony with the experiments of Loeb, Budgett, Coehn and Barratt, a tropism at the anodal side of the neuroblast and that the KCl constituents of the neuroblast gathering on this side thus increase (besides its conductivity) the anodo-tropic character of its colloidal substance. This anodo-tropic character of the colloidal substance of the axis-cylinder is, moreover, in harmony with Hardy's experiments on the kataphoresis of albuminoids.

Considering the fact, that the kataphoresis which genuine albumen and lecithin show is already generally an anodic one (Höber, loc. cit.) it is clear that the additional composition of the neurone and its surroundings still favors this, since the colloid particles of the young axon are embedded in a medium containing a quantity of KCl, that makes its preponderating reaction alkaline. Moreover the greater conductivity which KCl gives it, may cause the greater quantity of electricity to be led through it.

That the constituents of a peripheral nerve are strongly conveyed to the anode is also experimentally shown by Hermann, to whose experiments I return later (see p. 291).

From every standpoint indeed it seems that the conditions for the primary outgrowth of the axon along with the kathodic current to the anodic field have been realized in the nervous system.

chen. Forschung. Ergebnisse der Physiologie von Asher und Spiro, Jahrg. VII, 1908.

Alcock and Lynch. On the relation between the physical, chemical and electrical properties of the nerves. Part IV: Potassium, chlorine and potassium chloride. Journal of Physiology, vol. 42, 1910.

Macallum. Surface tension and vital phenomena. University of Toronto Studies, No. 8, Physiological Series, 1912.

The outgrowth of the axis-cylinder begins in the chick embryo about the second day of incubation (Bok).

Not until much later—according to Cajal when the growth tip of the axis-cylinder has reached, or nearly reached, its end point (about the 6th day of incubation in the chick embryo, Bok)—does an outgrowth of the dendrites begin, which make their way in the direction of the stimulus, that is in the direction of the kathode.

THE FORMATION AND CONTRACTION OF DENDRITES. THE FINAL SHIFTING OF THE PERIKARYON

I believe that there is a principal difference biologically as well as biochemically between the anodic elongation of the axon and the kathodic tropism of the dendrites.

The primary growth of the axon is in the beginning not directed to a certain point, but merely from a certain kathodic center, the outgrowth of dendrites, however, is much more influenced also in the beginning by their final end-points.

Their tropism corresponds with the regular appearance of the law of stimulation of protoplasm and exhibits a kathodic character, probably related with a more advanced nervous function for which a further stage of development is necessary.

This kathodic growth direction, as well as the kathodic taxis, is the usual thing in nature and, as Loeb and Maxwell have shown, is in harmony with Pflüger's law. We only have to prove that there are no factors which might interfere with it and change it into an anodal elongation.

This question is the more important since it may be that in the first phase of outgrowth of dendrites, which is not yet accompanied by a secondary shortening of the dendrite and the shifting of the perikaryon, a kataphoretic process might introduce it or at least be involved in it. Anyhow, the kataphoretic qualities of the dendrites may never be such that they should counteract the kathodo-tropic process which certainly is the chief factor in the shortening (contraction) of the dendrite and the shifting of the cell.

of Loeb and Budgett that the kathodic tropism, following the law of irritation, is chiefly dependent on *intra*-cellular protoplasmatic conditions and that the extra-cellular medium does not act such a part here as it does in anodal extensions.

Indeed, it seems more probable that the later outgrowth of the dendrites as well as their secondary contraction, including the shifting of the cell body is a process different in principle from the anodal outgrowth of the axis-cylinder, a process for which a greater functional completeness of the neurone is necessary, and that we may only say that the character of the chemical constitution of the dendrite is not such that it would interfere with it by a disturbing anodal process.

There are still three questions that may be mentioned in this discussion.

MONOAXONISM AND POLYDENDRITISM

The first question is why only one axis-cylinder leaves the cell, one which becomes complicated only by collaterals which proceed perpendicularly from it during its course, while from the cell-body, a large number of dendrites may and generally do grow out to several centers of stimulation (monoaxonism and polydendritism).

To explain the monoaxonism we may first consider what would happen if two kathodic currents traversed the young neuroblast at the same time. In a purely polar tropism, as galvano-tropism preëminently is, it is a familiar feature that the object under the influence of the current places itself so that the influence is equally great on both sides of the object. Only then does the state of equilibrium begin.

J. Loeb[15] in particular has shown this repeatedly, for example in his 7th Lecture, in which he speaks of radiating energy and heliotropism, and points out that the orientation of a simple object will continue until all its parts lie at the same angle with reference to the influence.

[15] J. Loeb. Vorlesungen über die Dynamik der Lebenserscheinungen. Leipzig, Joh. Ambr. Barth. 1906, p. 171.

As long as the influence on right and left, or indeed on all sides, be unequal, the object will change its position until the state of equilibrium is arrived at and the influence is the same everywhere.

Let us now apply this to a charged field from which a stimulus irradiates and passes to a cell in the neighborhood.

It will be clear, without anything further, that the outgrowth of the axis-cylinder in the current from the kathodic field to the anode has a state of equilibrium only in the course, that is, lengthways, of the current, i.e., in a collateral growing out perpendicularly or, where the growth stimulus proceeding from the irradiating current activated a cell in its neighborhood, the latter must send out its axis-cylinder also perpendicularly from the source along with the current. This explains the peculiar fact of collaterals of axis-cylinders in the commencement of their course having invariably a strictly perpendicular position with regard to the axis-cylinder.[41]

The irradiation current which radiates sideways from an activating axis-cylinder must naturally move in a direction perpendicular to this axis-cylinder. This is a physical fact that is only changed at the growing point of the activating axone.

It will thus be seen that the presence of one axon, as well as the perpendicular position of the collaterals on the axis-cylinder, are but natural consequences of the perfect bipolar character of the current. Now the same holds good if two or more differently running tracts, or differently placed centers, activate one cell simultaneously. We then may also expect only one axis-cylinder in the resultant line of the two current directions (two bio-electric fields), since only in this line the equal influence on both sides of the growing point, the energetic equilibrium, is realized.

What will be the case if two or more activating centers are present not acting simultaneously? One of these activating centers has to be the first and causes the initial outgrowth.

[41] At the time when coloration and impregnation methods were not so advanced as now, the differential diagnosis of collaterals and dendrites was sometimes made on account of the perpendicular position of the latter on the axon

If, however, an axis-cylinder has started to grow, we may expect that the favorable conditions which it offers for the current, on account of its greater conductivity, are such that the obstacle to the formation of a new axon at some other place is so much greater, that the current will take the present path of enlarged conductibility, the course of which it may influence perhaps without, however, causing a new axon to grow out, the point of application of forces being localized.

The conditions with the dendrites are quite different.

This process is by no means necessarily limited to one part of the surface of the cell since its whole body containing Nissl substance is equally sensitive and any stimulation may cause protoplasmatic shiftings in their direction, whereby the principal dendrite and finally the shifting of the cell-body itself will doubtless take place in the direction of the maximal stimulus.

In other words, if another stimulus than the one which formed the axis-cylinder reaches the cell, it will form no new way out, since this would require more energy than a following of the present path of greatest conductivity, but a new stimulus coming from another center, may produce—or even must produce—a new dendrite. Since the perikaryon is equally sensitive (except the axon hillock) to it everywhere and since already existing dendrites are not in its path, the nearest cellular or dendritic surface will be the point of application for its influence, i.e., for the formation of a new dendritic outgrowth.

THE SELECTIVITY IN THE PROCESS OF NEUROBIOTAXIS IN HARMONY WITH PSYCHOLOGICAL LAWS

I now come to the second and most important point in the tract formation, that which determines the selectivity of the definite connections.

It has escaped the observation of all the earlier investigators that the selectivity of the tract formation depends upon simultaneous, or better, correlative, stimulation. Cajal assumed chemical secretions coinciding with stages of evolution, also ascribing an influence to the glia cells in the secretion of such "substances attractives" and *without pointing out by which factors*

these stages of evolution were defined, which he could not do since his conclusions were chiefly, if not solely, based on ontogenetic, that is engrammatic observations. Held speaks of a "Prinzip der Auswahl," upon the character of which he does not enter, and with regard to his own researches Harrison[42] justly remarks:

> There is nothing in the present work which throws any light upon the process by which the final connection between the nerve and its end-organ is established.
>
> That it must be a sort of specific reaction between each kind of nerve fiber and the particular structure to be innervated seems clear.

That the relationship for the final connection, which holds good in the central nervous system for the dendrites and the cell-shifting as well as for the axis-cylinders exists in the correlative, mostly synchronous stimulation condition of the elements, I first deduced from the selective character of the cell shifting, and this could be further clearly demonstrated by the axonic connections existing in the nervous system. It even explains a series of peculiarities in the course of the fiber tracts which otherwise confronted us as constant but inexplicable facts, especially in the so-called central motor tracts such as the pyramids.

This fundamental law of neurobiotaxis shows us not merely that the fundamental law of association in psychology is at the same time an anatomical law, but also how wonderfully polar the whole character of tract formation is, an how it therefore falls within the range of the galvano-tactic and galvano-tropic phenomena.

In order to explain this phenomenon of selectivity in an electro-chemical way, I must draw attention to the following points.

It is presumed that the presence of potassium salts has the peculiarity that it greatly increases the conductivity of the axis-cylinder for the electrolytic current.

There is even an inclination to ascribe the strong conductivity of the axis-cylinder, as compared with the synapse, to the high percentage of potassium salts in the axon (MacDonald, Macallum).

[42] Harrison. The outgrowth of the nerve fiber as a mode of protoplasmic movement. Jour. Exp. Zoöl., vol. 9, 1910, p. 787.

turb a count quite considerably. What are the characteristics of stage 6? Standing at the transition point between the shrunken hyperchromatic Hodge stages and the following hypochromatism and upset of the nucleus-plasma relation, it has a more swollen, vesicular and disproportionate nucleus than the resting type, though its plasma now comes to show the average distribution of chromatic substance of that type. I have pointed out several times that unless its nuclear size and appearance be kept in mind, it will be mistaken for a resting cell.

A second point: Stage 13 is one of complete basic dechromatization. The Nissl substance is gone, likewise the nuclear chromatin. The rapidity of such dechromatization depends on the relative differentiation. It may appear within a few hours in the Purkinje cell, though probably not unless the animal is advanced in activity to start with. Not only has it never come under my observation in a lower type of cell within the time necessary to produce it in the cortex, but the indications have always been that the lower cells at this time were many stages removed from exhaustion. It was only marked, though still not absolute, after two weeks of continuous excitation of the crayfish cell.

Yet Kocher is extremely liberal with stage 13. He always finds it in the cervical and lumbar cord cells, and in two cases out of the four animals counted there are more than from the cerebellum. Not only do I regard this as impossible on the basis of differentiation, but it does not jibe with the text, for he only mentions grades of plasmic chromatolysis, which obviously is another thing from nuclear plus plasmic dechromatization. Nuclear *dechromatinization* would exact a comment from any one. In other words, some at least of the stages identified as exhaustion are fairly doubtful, and this carries closely related stages. One is forced to the same deduction for Kocher's whole table 3.

It is the sort of rebuttal of a criticism that personally is very distasteful, for it carries the possible imputation that the originator of said stages is the only one competent to pass judgment upon them. This is not true, for eight students who have worked with me have had no difficulty after several months

study in separating them as well as myself. As Kocher denies "progressive changes in the morphology of the cells," it is evident that he missed the finer points essential to a differentiation.

Outside of these technical points, no denial of the existence of quantitative differences can be made on the comparison of four animals. The range of individual variation is too great. There is no way of telling what the state of activity with which the experiment begins. Kocher's control animal may very well have been two or three times as functionally advanced as the one exercised the most was to begin with. I have seen several undisturbed animals who showed a degree of activity almost as great as one subjected to exhausting overstrain. The control comparison method, though valuable and frequently the only resource, affords no absolute deductions, unless all conditions are certain. Apparent inconsistencies, of which I have encountered many, one by one have cleared up as all conditions became known.

Just for one example, age is a factor. Very young animals usually show a hyperactive state as compared to the adult. Resting and early active cells may be absent in section after section. Very probably this is the reason why Kocher's three month old puppies showed "no discoverable differences in staining reaction."

One final rejoinder concerns a matter, which, though even more distasteful, I refuse to pass over. In April, 1910, I published the results of 2200 cell measurements. Even in the preliminary communication of November, 1909, on normal functional activity, which Kocher cites, the results from 1500 of these measurements were stated, which explicitly did not include those previously published from the shock and hemorrhage series. Further, in the same paper the results of differential counts of 3,600 cells were included. In still earlier communications, of April and July, 1909, on shock and hemorrhage respectively, which he also cites, it was made sufficiently clear that preliminary counts of 1300 and 1200 cells had been made, as it was stated that 100 cells were counted in each experiment.

From this brief survey, it may be imagined with what pained surprise one reads from Kocher, "Obviously the observations

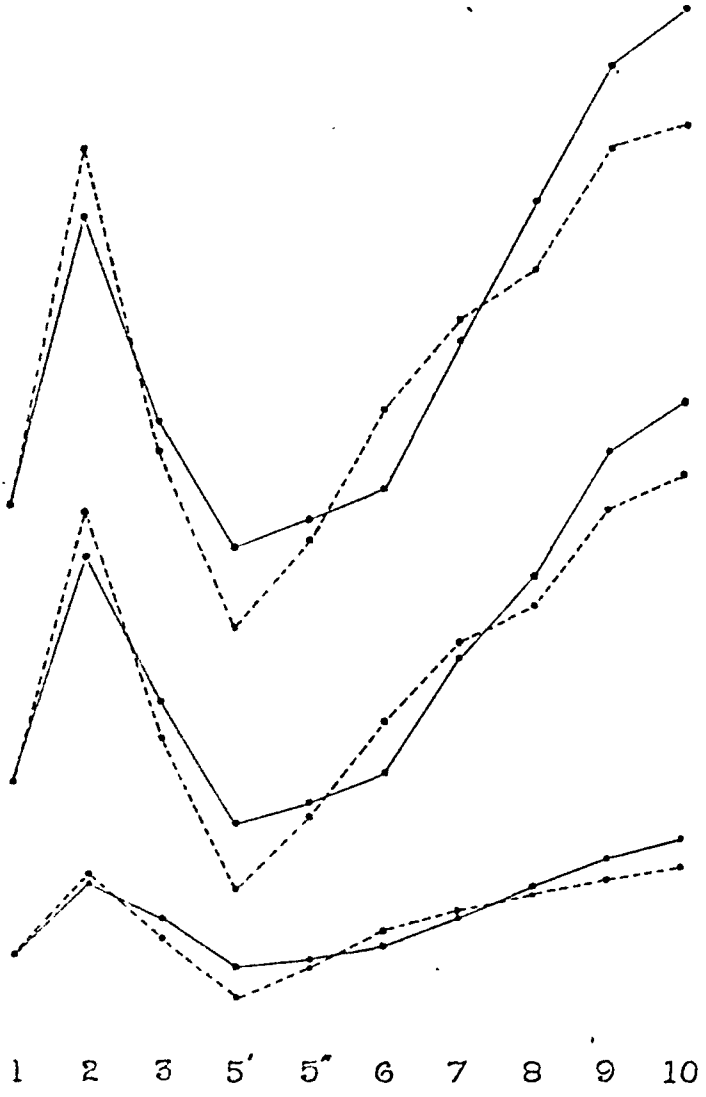

Fig. 2 Size relations of function compared by the three methods.

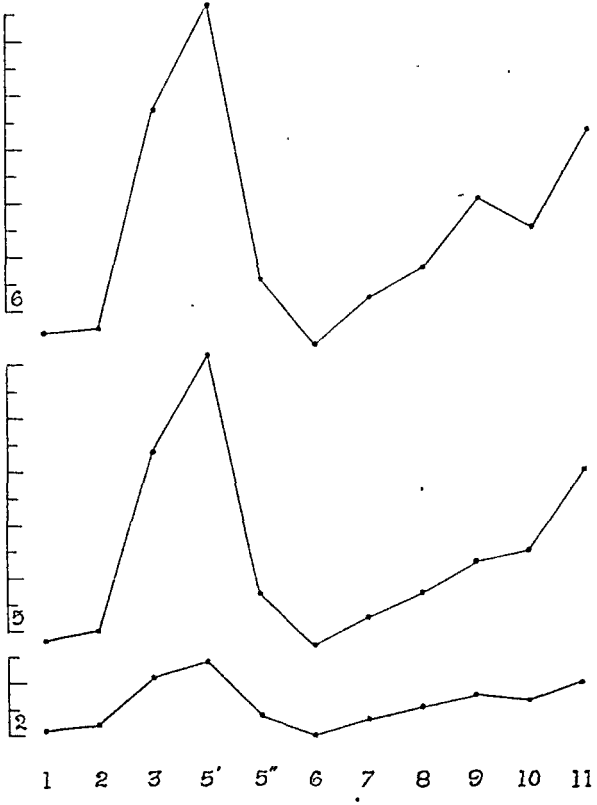

Fig. 3 Nucleus-plasma relations of function compared by the three methods.

been made in previous work ('14, p. 494), and more strongly supported in some unpublished work, that function is the sole determinant of absolute size. Non-divisional growth in mass is a functional growth. Here are two animals born together and living under functional conditions as identical as may be. Their cells show the same absolute size. It is a noteworthy verification of the deduction.

It fits in with this relation of function to size that the evidence is accumulating of a tendency to a uniformity of absolute size among corresponding nerve cells of animals of the same species. When sufficiently demonstrated it would be understandable on the basis of average general functional usage. The exceptions thereto so far in the dog, the unusually large cell, have been associated with a known history of unusual training and activity. It makes the nerve cell agree with Conklin's conclusion that within the same species cell size is approximately constant. Making simply a statement here of the probable principle, it is to be noted that these two dogs, being not yet grown, offer no evidence for or against species uniformity of absolute size, save that they are progressing together under identical conditions.

It might be expected and to some extent it is true that all stages succeeding stage 1, being based quantitatively upon it, might show this same correspondence of absolute size. However, in all stages except stage 1, one encounters a shifting range of size throughout the stage. The results will vary according as the majority of cells are at one end or the other, or well distributed in the chance of a section. Stage 1, though there are intermediate grades to stage 2, was frankly selected in both animals as the nearly flat type, with this very point in view, and intermediate stages were thrown to stage 2.

THE INCONSTANCY OF COLLECTIVE AVERAGES OF FUNCTIONING CELLS

It only remains to demonstrate from the data in table 3 the inconstancies which may result from averaging all cells irrespective of their functional state, and to expose the fallacy of deny-

ing on such a basis the existence of functional size changes. For the inconstancies, take the control data: The average area of thes mallest cell, stage 5'—and the average covers its own variations—is 14.57, that of the largest is 27.72 or nearly double; the average volume of the smallest cell is 70.44, that of the largest 200 or nearly triple the size. In the exercised animal with the still larger stage 11, the largest volume is nearly quadruple the smallest, and its area again more than double. Is it not apparent to any one that if such widely variant sizes or areas are averaged, the result depends upon the particular distribution of types and that a wide range of results is possible? If, out of 20 cells, even in area computation, 5 measure 14 sq. cm. and 15 measure 27, the average is 24, whereas if 15 measure 14, and 5 measure 27, the average is 17 sq. cm. The results may or may not prove anything about the immediate functional state.

One can then with fair probability explain what did happen in Kocher's case. He finds only small variations in average area size between control and exercised animals and these not constant. So far as different functioning stages appear, they tend to be distributed rather than bunched. A general average, taking into account the smaller range of variation of area figures, would tend to equalize the differences due to unequal distribution of various-sized types.

So Kocher, having smoothed out individual cell variations by averaging, found no great difference between an animal and its control. His results are just what might be expected in probably the majority of cases, and instead of confounding the writer in respect to functional size changes, tend only to support the induction previously stated of a uniformity of cell size as a general rule for a species. Were it not for this tendency to equality of size of corresponding cells, collective averaging would not have afforded so many positive results as it has.

Since the method of collective averaging is the one which has been always used, how about such results as those of Hodge? Are they discredited? No, but they must be qualified. Hodge found a smaller size in the stimulated spinal ganglion as compared with the unstimulated simply because there were enough

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THE FOREBRAIN OF ALLIGATOR MISSISSIPPIENSIS

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From the Anatomical Laboratory of the University of Chicago

FORTY-SIX FIGURES

CONTENTS

The granule cell layer shows a wide range of types among its neurones. The following types, based on a study of Golgi preparations, have been distingushed among the granule cells of the bulb.

1. Intrinsic or type II cells. These neurones have small cell bodies, with dendrites that are short, thorny, and branching, and which pass out in every direction from the cell body. No axones can be distinguished. These cells are intrinsic neurones, serving for the correlation of impulses within the layer. Some of the smaller stellate cells appear to serve as intrinsic neurones, at least so far as can be judged from the material studied.

2. Stellate cells (figs. 27, 28). These are similar in appearance to the cells so named by Sheldon in the teleostean olfactory bulb. The cell bodies are angular or somewhat star-shaped as the name indicates. The dendrites are thick and thorny and many branched and extend out towards the periphery of the bulb. In the plexiform layer they interlace with the dendrites of the mitral cells and of the goblet cells. Some of the dendrites extend outward into the glomerular layer but it was not determined whether these dendrites actually entered into the formation of glomeruli, as Sheldon ('12) found to be the case in the teleosts. The axones in many cases form synapses with branches of the mitral cell dendrites. Sometimes they enter the tractus olfactorius, although they have been followed no great distance in it. Some of the smaller stellate cells do not send their dendrites outward beyond the cell bodies of the mitral cells. Furthermore the axones of such cells often end about other cells of the bulb and so serve as intrinsic neurones.

3. Goblet cells (figs. 25 to 27). These are large, oval cells whose dendrites are similar in appearance to those of the stellate cells. Sometimes the dendrites of the goblet cells reach the glomerular layer, and have been seen entering into the formation of a glomerulus. In other cases, the dendrites of the goblet cells do not enter the glomerular layer but are dependent upon the mitral cells for their stimulation. The axones of the goblet cells enter the tractus olfactorius, at least in some cases.

From the standpoint of their types of synaptic connection apparently three functions are served by the neurones of the granule cell layer. The first of these is that of diffusing and summating the incoming olfactory impulses and so strengthening the discharge into the hemispheres. This purpose is served by the type II cells, the stellate cells, and, in part, by the goblet cells (particularly those found in the anterior part of the bulb). All these cells receive their impulses by way of the mitral cells and do not send their axones into the tractus olfactorius.

A second group of these stellate and goblet cells send their dendrites into the glomeruli and their axones into the tractus olfactorius and so, from a functional standpoint, are practically mitral cells.

The third function served by neurones of the granule cell layer is that of acting as the cells of secondary olfactory nuclei. Such cells receive impulses from the mitral cells and send their axones into the tractus olfactorius. The goblet and stellate cells offer examples of this type of neurone.

Judging from what is known of the development and specialization of the centers of the central nervous system, it seems but fair to suppose that, in phylogeny, the centers of the olfactory bulb arose from undifferentiated central gray. Johnston ('98) has shown that in Petromyzon and in Acipenser, neurones of this mitral cell type are found all through the central gray. The same author ('15) has described, in Cistudo carolina, a granule cell layer in which are cells functioning as mitral cells.

Certain cells of the central gray (on the whole those nearer the periphery) will receive a larger number of the incoming olfactory impulses. Under the operation of neurobiotaxis (Kappers, '14) such cells will be drawn toward the periphery and, in this way, a mitral cell layer will be formed. Accompanying such a migration toward the surface and the consequent higher specialization, there will be a differentiation in form and in size to meet the greater demands.

Not all the cells left in the central gray will lose their connection with the fila olfactoria and so certain goblet cells and probably some of the stellate cells (although the proof for this is not

Nucleus preopticus (figs. 9, 10, 18 to 21). This term has been applied to the cell mass which appears in the region of the preoptic recess just in front of the level of the commissures and which extends caudad still occupying this position. It passes over into the hypothalamic region with no definite line of separation between the two areas. The nucleus preopticus receives impulses from the stria terminalis, from fibers of the medial olfactory tract which have decussated by way of the anterior commissure, and, at its anterior end, from some few fibers of the tract of the diagonal band of Broca.

Interstitial nucleus (figs. 10, 19, 20). Cajal ('11, vol. 2, p. 723) described this nucleus and figured it in the mouse, calling it "noyau interstiel de la voie de projection de l'écorce temporale." He says further "Malheureusement, il ne nous a pas été possible de déterminer de façon précise les relations qui existent entre la bandelette semi-circulare et ce noyau, et cela à cause de la rareté des bonnes imprégnations. Ajoutans que cet amas de la région sousthalamique pourrait fort bien être encore un ganglion moteur."

Johnston ('15) has described the olfactory projection tract of Cajal for the turtle but has said nothing of the interstitial nucleus. In the alligator the nucleus appears in the preoptic region near the posterior end of the hemisphere as a ridge of cells extending lateralward in close relation with the nucleus ventro-medialis, arching dorso-medialward above the forebrain bundles and extending medialward into relation with the more dorsal part of the mass of the preoptic nucleus. It extends caudad throughout the preoptic region but in the hypothalamic region is gradually replaced by the hypothalamic nuclear ridge.

Part of the fibers of the olfactory projection tract of Cajal arise from the ventro-medial nucleus. Cajal ('11, vol. 2, p. 725, fig. 463) has shown some of the neurones of that nucleus giving rise to these fibers. Many of the fibers having such an origin quite probably send off collaterals among the cells of the interstitial nucleus. Part of the fibers of this olfactory projection tract arises from the interstitial nucleus. The tract passes caudad with the fornix into the ventral part of the hypothalamus.

Nucleus of the diagonal band of Broca (figs. 8, 9, 17). This nucleus was first described for reptilian brains by Johnston ('15) in the turtle, Cistudo carolina. It is present in the alligator in practically the same relations as in the turtle. It appears behind the level of the tuberculum olfactorium as a dense collection of cells arranged in a cortex-like layer in the ventro-medial angle of the hemisphere. It extends dorsalward along the medial surface as a somewhat less dense, cortex-like layer which comes into relation with the medial parolfactory area and cannot be sharply distinguished dorsalward from the cell mass of the primordial hippocampus. It extends from the ventro-medial region lateralward, as scattered clusters of cells, into relationship with the nucleus of the lateral olfactory tract. The nucleus of the diagonal band extends posteriorly just outside of the medial forebrain bundle into the region of the preoptic nucleus. It is accompanied, as in the turtle, by bundles of fibers which serve for connecting the lateral and medial olfactory areas. The writer is particularly indebted to Dr. C. J. Herrick for aid in identifying this nucleus in the alligator.

Basal nuclei of the lateral wall. Students of the reptilian brain have generally recognized two basal centers in the lateral wall of the cerebral hemisphere, the corpus striatum and the epistriatum, and some have recognized a third region distinct from both of these, comparable with the mammalian nucleus amygdalae. According to these observers, the epistriatum is the more dorsal member of the complex and is in continuity with the cortical lamina. The extent of this continuity varies in different reptiles, depending upon the species and the general form relations of the hemisphere, particularly upon the ventro-lateral extent of the ventricle. In Testudo graeca, DeLange ('13a, p. 113, fig. 8.) has shown that the epistriatum is continuous with the lateral or pyriform lobe cortex throughout its whole extent. Kappers and DeLange consider the epistriatum to be striatal in origin and to have acquired secondarily a connection with the cortical lamina. They consider the epistriatum an olfactory nucleus of the second order and the entire epistriatum complex the homologue of the mammalian nucleus amygdalae.

visceral centers, a lateral group which is the place of termination for the somatic impulses brought in by the optic and lemniscus systems and, intermediate between these two groups, a third nucleus which receives fibers of both the visceral and somatic type. This nucleus is the nucleus medialis or the nucleus rotundus of some authors.

In the medial group are the nucleus anterior and the nucleus magnocellularis. The nucleus anterior (figs. 10 and 11, 20), as its name implies, lies at the very anterior end of the thalamus. It is dorsal in position and its cells are smaller and more closely packed together than are the cells of the lateral nucleus. It receives fibers from the hypothalamus and is connected with the small celled ventro-medial part of the hemisphere by means of a fiber tract.

The lateral group includes the nucleus lateralis, a special derivative of this nucleus—the pulvinar—and another optic center which most writers have termed the corpus geniculatum laterale. The nucleus lateralis is conspicuous because of the large size of its neurones. The cell bodies of these neurones (figs. 41, 42, 43) are large, goblet or triangular in shape, and have thick thorny dendrites which extend out in every direction from the cell bodies. The axones enter the lateral forebrain bundle. This nucleus is lateral in position, being lateral and somewhat ventro-lateral to the nucleus anterior and lateral to the nucleus medialis (or rotundus). It receives lemniscus fibers and some optic fibers and, with the lateral thalamic optic centers, represents the beginning of the neothalamus (Edinger) of higher forms, i.e., that lateral portion of the thalamus which serves as a place of synapse for nervous impulses passing to the neopallium and which develops parallel with the development of the neopallial cortex. In the more posterior part of the thalamus, a lateral portion has begun to differentiate away from this nucleus and to form a beginning of the pulvinar. This separate nucleus is developed under the direct influence of the incoming optic fibers.

There are other cell masses in the thalamus proper, as for example the nucleus reuniens figured in the alligator brain by

DeLange ('13); but the writer knows too little of their relationships or significance at present to discuss them.

Hypothalamus. The hypothalamus of the alligator is highly developed. An examination of figures 11 and 12 will show that a number of cell groups are present. In his 1913 paper DeLange has named these different groups. No attempt has been made to do so in the present paper because of a lack of knowledge of the fiber connections of the different groups.

FIBER CONNECTIONS

With the foregoing descriptions of the cell groups as a basis, attention can now be turned to the courses and terminations of such of the fiber tracts as have been worked out. Papers published by C. L. Herrick, Edinger, Adolf Meyer, Kappers, Unger, DeLange, and Johnston contain descriptions of the fiber connections of the reptilian brain. These descriptions in almost every case, have been based on adult material, the work being done with Weigert preparations which bring out the myelin sheaths. On the other hand, the work for this paper has been done chiefly with Cajal and Golgi material, which bring out the unmyelinated fibers and, in many cases, the axis cylinders of the myelinated ones. Repeated attempts to prepare a series stained by the Weigert method were not successful so far as the forebrain was concerned. These failures, of course, may have been due to faulty technique, but only extremely young material was available and in such material many of the myelin sheaths may not have become mature. C. J. Herrick ('10) has figured on pages 537, 539, and 541 some cross sections of the forebrain and the thalamus of Alligator mississippiensis showing the fiber tracts and the positions of some of the centers. These drawings were made from Cajal material and were of much help. A series stained with Ehrlich's haematoxylin and an imperfect series prepared by the Leuden van Heumen method were used to check the results obtained by the Cajal method.

representatives of that tract in reptiles. It has been termed in this account, the ventral olfactory projection tract.

The portion of this tract which arises from the pyriform lobe and associated regions is evidently the same tract as that described by Kappers and Theunissen ('08, p. 225) for the lizard, Iguana, under the name tractus olfacto-habenularis (see figures 21 and 22 of their paper). Farther forward these authors describe it as turning lateralward to connect with the 'lateralen Lobusrinde' (fig. 20), which is apparently the pyriform lobe region of the present account.

There are probably other components of this fiber complex which have not been impregnated in the preparations studied.

3. *Tractus cortico-habenularis lateralis posterior* (figs. 20, 21). This large system of fibers arises from the nucleus of the lateral olfactory tract and the ventro-lateral part of the dorso-lateral area. Some of its fibers may arise from the overlying cortex of the pyriform lobe. These fibers pass medialward, at the same time sweeping dorsalward to avoid the area of distribution of the stria terminalis. At the lateral border of the thalamus they run parallel with and dorsally of the stria terminalis fibers (figs. 20, 21) and here they turn abruptly dorsalward to enter the stria medullaris thalami.

4. *Tractus olfacto-habenularis medialis* (figs. 20, 21). This tract arises from the more posterior portion of the nucleus preopticus, runs dorsalward medial to the medial forebrain bundle and turns forward and forms the most anterior part of the stria medullaris.

5. *Tractus olfacto-habenularis lateralis* (figs. 20, 21). This tract has its origin from the more anterior portion of the nucleus preopticus. It runs first lateralward on the extreme ventral surface of the brain ventrally of the basal forebrain bundles, then backward and dorsalward, joining the tractus cortico-habenularis lateralis anterior in the angle between the ventro-medial nucleus and the lateral forebrain bundle and passes dorsalward with it to enter the stria medullaris. (See description of tractus cortico-habenularis lateralis anterior for a further account of the relations.)

and the preoptic portion of the stria terminalis. Some of the fibers arise probably from cells of the interstitial nucleus and fibers from cells of the ventro-medial nucleus probably send collaterals into the interstitial nucleus. The fibers of this great olfactory projection tract as they swing medialward come into relation with the descending fibers of the columna fornicis and there turn sharply caudad and run with the latter bundle backward, medialward and ventralward to the mammillary body (figs. 20, 21). (Dr. C. J. Herrick first called the writer's attention to the fact that fibers of this olfactory projection tract join the fornix fibers and accompany them ventralward).

Ramón y Cajal ('11, vol. 2, pp. 722–723, fig. 462) has described and figured this tract and its associated nucleus in the mouse. Johnston ('15) described the tract in the turtle. He considers it to be the characteristic connection of his medial large celled nucleus of the amygdaloid complex (the ventro-medial nucleus of this description), but does not mention the interstitial nucleus which accompanies it.

Basal forebrain bundles

Medial forebrain bundle (figs. 9, 16, to 21, *M.F.B.*). This is the tractus septo-mesencephalicus of Unger and DeLange. It arises from the parolfactory (septal) nuclei and runs, accompanied by fibers of the fornix longus, medialward and ventralward until it meets the lateral forebrain bundle, which lies farther laterally. The two bundles can be distinguished from each other for a long distance because of a difference in the angles at which the fibers are running. Finally the two become closely mingled and it requires careful study to distinguish them, although such a differentiation is quite practicable. According to DeLange ('13) and Unger ('11) the medial forebrain bundle runs to the midbrain. In the alligator in material prepared by the Cajal method a part of the fibers appear to end in the hypothalamus (tractus olfacto-hypothalamicus of the literature), while others pass caudad to the midbrain (tractus olfacto-pedunculares).

There is no direct evidence in the material studied regarding the direction of conduction, but the probability is that impulses

pass in both directions. It serves, then, partly as a discharge path from the parolfactory areas (tractus parolfacto-hypothalamicus and tr. olfacto-peduncularis) and perhaps also from the tuberculum olfactorium, and partly as a pathway by which visceral impulses from the hypothalamic region may reach the medial parolfactory area (tractus hypothalamo-parolfactorius) and, either with or without a synapse there, the hippocampus. Fibers connecting the parolfactory areas and the hippocampus run on the medial and lateral borders of the medial forebrain bundle.

Lateral forebrain bundle (figs. 9, 16, to 21, 37, 45, 46, *L. F. B.*). This bundle is made up in part of axones arising from the projection cells of the striatum. It runs ventro-medialward, joins the medial forebrain bundle on its lateral side, and then passes caudad into the diencephalon. This is the tractus strio-thalamicus of DeLange and Unger.

Besides these components of the lateral forebrain bundle which carry impulses from the striatal region, there are fibers from the lateral and medial nuclei of the thalamus which run ventralward and join the other fibers of this bundle and then go forward to the striatum. These facts are known because axones or the cell bodies of the lateral nucleus and nucleus rotundus have been seen to join this bundle (tractus thalamo-striaticus, or thalamic projection tracts).

There is a second thalamo-striatal path which runs from the anterior nucleus of the thalamus to the ventro-lateral small celled part of the hemisphere (that part which is Johnston's nucleus caudatus). This has been described by Johnston, DeLange, and others.

GENERAL DISCUSSION

The problems of forebrain morphology and especially those dealing with the evolution of the cortical areas have always had a peculiar fascination for the comparative neurologist. The broad lines and many of the details of forebrain development throughout the vertebrate series have been brought out by such observers as Edinger, Elliot Smith, Johnston, Herrick, and Kappers.

It is with considerable hesitation that the writer has undertaken the analysis of the anatomical data given on the preceding pages. Insufficient time and knowledge and the lack of experience have been very clearly realized and the following statements are offered merely as suggestions or as possible interpretations of some of the changes occurring and the factors operating during forebrain evolution.

Following the type of interpretation of Edinger, Herrick, Kappers, and Johnston, centers of the alligator hemisphere may be classified under two general heads which may be subdivided as follows:

I. Centers dominated by olfactory impulses
  A. Basal centers
    1. Medial olfactory area
      Nucleus olfactorius anterior (in part)
      Nuclei of the septum (in part), or parolfactory nuclei
    2. Lateral olfactory area
      Pyriform lobe complex (in part)
      Amygdaloid complex (in part)
    3. Intermediate olfactory area
      Tuberculum olfactorium
      Nucleus olfactorius anterior (in part)
      Nucleus of the diagonal band
    4. Correlation centers between telencephalic and diencephalic regions
      Tuberculum olfactorium (in part)
      Parolfactory nuclei (in part)
      Nucleus commissuralis hippocampi
      Bed nucleus of the anterior commissure
      Nucleus preopticus
      Interstitial nucleus of Cajal
      Amygdaloid complex (in part)
  B. Cortical centers (archipallium of Edinger)
    1. Hippocampal formation
      Small celled non-laminated part of hippocampus (the primordium hippocampi of Johnston, '13 and '15)
      Dorso-medial cortex (primordial gyrus dentatus, Elliot Smith, '95, Meyer, '92, Levi, '01)
      Dorsal cortex (hippocampal cortex, subiculum of Johnston '13)
    2. Lateral cortex (pyriform lobe)
    3. General cortex (to some slight degree)
II. Centers dominated by ascending somatic impulses from the thalamus
  C. Basal centers
    1. Dorso-lateral area
    2. Intermedio-lateral area
    3. Ventro-lateral areas (comparable to corpus striatum of Johnston '15)

D. Cortical centers
1. General cortex (in part)
Primordial general cortex (a special portion of this area in close relation with the dorso-lateral area)

The basal olfactory centers of the telencephalon will be seen to be separated into two broad groups. In the first group are those of the medial, intermediate and lateral areas which serve primarily as secondary olfactory centers. These are old in type, having their representatives in the hemisphere from cyclostomes (Johnston, '12, Herrick and Obenchain '13) up through the vertebrate series to man. They were originally simply a place of synapse and consequent redistribution of incoming olfactory impulses.

The second group of basal olfactory centers includes those which have developed within the hemisphere later in the phylogenetic history as a place of correlation between olfactory and non-olfactory impulses. It is significant that some of the centers (as for example the tuberculum olfactorium), judging from their fiber connections, are both secondary olfactory nuclei and correlations centers for olfactory and non-olfactory impulses. It is the forward growth, then, of non-olfactory fibers from the diencephalon into the secondary and tertiary olfactory centers of the hemisphere which has given the impulse toward differentiation to the telencephalon. These nuclei of the hemisphere, which serve as correlation centers for the olfactory and non-olfactory impulses, represent the beginning of that higher differentiation. Yet these basal centers do not form true cortex. In the Amphibia (Herrick, '10) in the ventro-medial part of the hemisphere, centers showing such type of correlation are present and the medial forebrain bundle, which opens the possibility of connection between the olfactory centers and the visceral centers in the hypothalamus, is well developed. In the dorso-medial part of the hemisphere of Amphibia the material, which is the primordium of the hippocampus, is present; it is under the influence of olfactory fibers and, to some extent, of fibers of the ventro-medial area of mixed function as just indicated. But here no clearly developed cortex is found and it is not until the

basal olfactory and non-olfactory correlation areas are well developed, as in reptiles, that true hippocampal cortex begins to appear.

Johnston has emphasized the fact that the hippocampus is an olfacto-visceral center, although in a later paper ('15, p. 412) he has said that there are olfacto-visceral correlations in the subiculum as well. It is well to notice that these types of nervous impulses are not first assembled in the hippocampus. On the other hand, this cortex simply brings together material already correlated, partly in the hypothalamus and more completely within the basal telencephalic centers. Three types of centers concerned with olfactory impulses are represented then within the hemisphere.

1. Those basal centers concerned with the distribution of olfactory impulses and their summation and correlation among themselves.

2. Those basal centers concerned with the correlation of olfactory and non-olfactory impulses.

3. Those centers which receive impulses from correlation centers of the second type or from similar non-olfactory correlation centers and integrate these impulses. This integration of material already correlated is characteristic of the reptilian cortex. Into the hippocampus come impulses from the parolfactory area and the tuberculum olfactorium on the one hand, and from the pyriform lobe cortex by way of the alveus on the other hand.

In Amphibia (Herrick, '10) the primordium hippocampi occupies the dorso-medial portion of the medial wall of the hemisphere. This region has all the characteristic fiber tracts of the hippocampus (cf. Herrick, '10, p. 480) but there is no differentiated cortex in this region except possibly to a small degree in Anura, where there is a row of cells close to the surface of the ventro-medial wall which send out wide spreading dendritic processes among the incoming fibers and which resemble in cell characteristics those cells found in the alligator at the anterior end of the hippocampal formation.

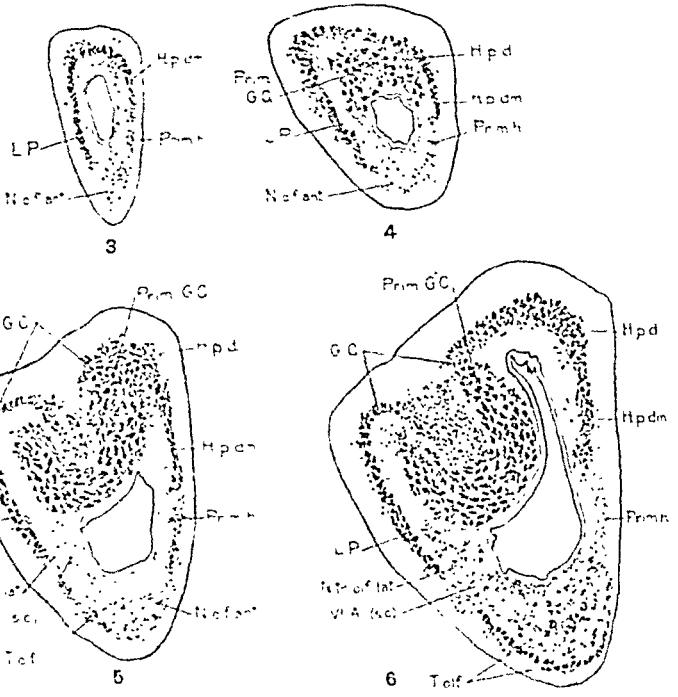
In lower reptiles the dorso-medial area begins to form true hippocampal cortex. In the turtle, although, as has already been

pointed out (see discussion of hippocampus), there is a clearly defined arrangement of a considerable part of the hippocampal formation into definite cortex-like layers, these layers have not moved out from the ventricle as in higher forms, but still form a ventricular mass.

The hippocampal cortex of the alligator represents another step in advance in differentiation, for here the cortex has moved away from the ventricle and accompanying this differentiation has been the specialization, at least to a considerable extent, of its medial aspect to serve as the afferent side of the cortex and its lateral aspect to serve as the efferent side. One of the causes at least, for the outward migration of cells of the dorso-medial area to form the hippocampal cortex is probably to be found in the operation of the law of neurobiotaxis (Kappers, '14). According to this law, cell bodies tend to migrate along their dendrites toward their source of stimulation. The medial olfactory tracts and other tracts bearing afferent impulses to the hippocampus are on the medial surface of the hemisphere and the cells of the developing cortical layers move out toward the surface of the hemisphere in order that they may come into closer relationship with the incoming impulses.

To recapitulate, the following steps appear to have lead from the primordial hippocampal type to the relatively simple type of cortex found in part of the hippocampal area in the alligator. In Amphibia (Herrick, '10) the afferent and efferent fibers spread out all through the dorso-medial area. Following a higher differentiation of the diencephalic and telencephalic subcortical correlation centers, there is a higher differentiation in the dorso-medial area so that the arrangement of the cells into cortex-like layers, such as we find in the turtle, occurs. This second step is followed in other reptiles by a further specialization of a part of the hippocampal cortex, so that it has an afferent medial side and an efferent lateral one.

The non-olfactory diencephalic fibers, which enter the telencephalon for the purpose of forming correlations with the incoming olfactory impulses, are partly visceral and partly somatic in type. Those ascending from the hypothalamus by way of



Figs. 3-12 A series of transverse sections through the hemisphere of Alligator mississippiensis. Toluidin blue. × 19. The serial numbers of the sections figured are appended to the descriptions.

Fig. 3 Section through the posterior part of the olfactory crus showing the anterior part of the pyriform lobe and the hippocampus (14 : 286)

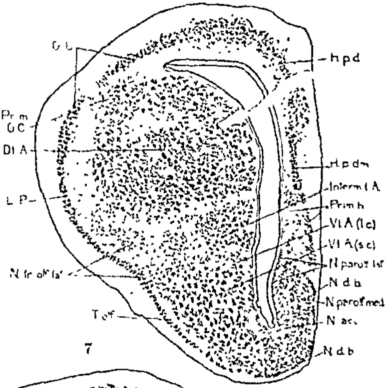
Fig. 4 Section slightly caudad to the preceding, showing the primordium of the general cortex (16 : 318).

Fig. 5 Section illustrating the characteristic appearance of the general cortex (18 : 353).

Fig. 6 Section somewhat caudad to the preceding (19 : 370).

Fig. 7 Section through the posterior part of the primordium of the general cortex, showing the basal nuclei of the lateral and medial walls in that region (22 : 416).

Fig. 8 Section slightly caudad to figure 7 (23 : 436).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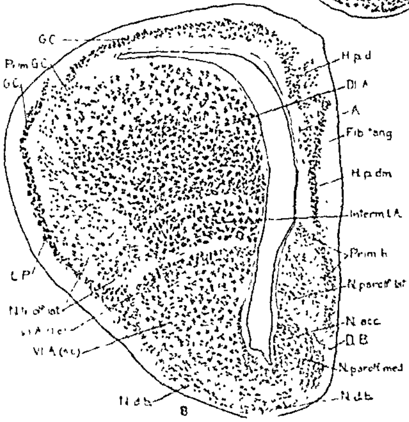
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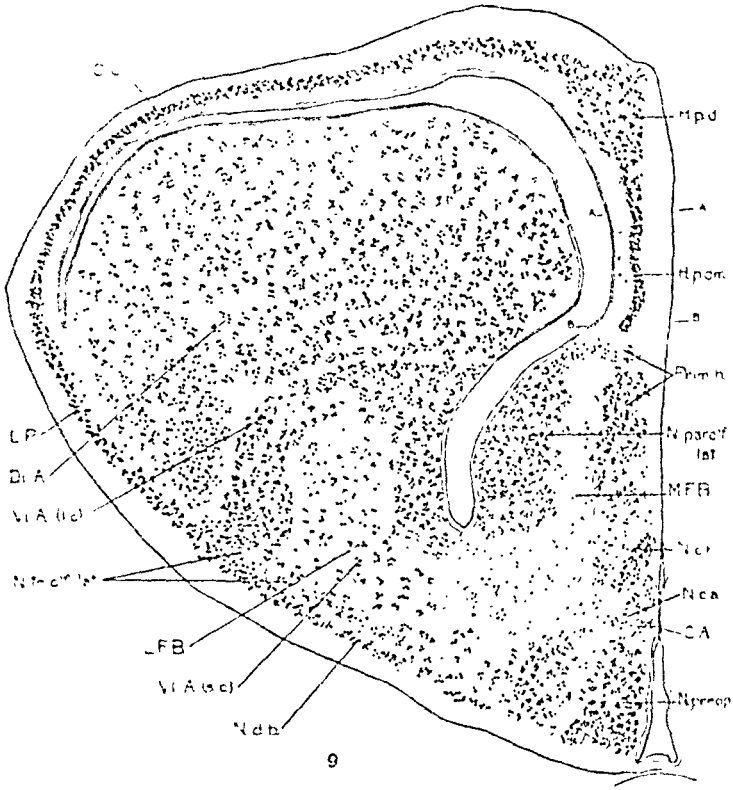
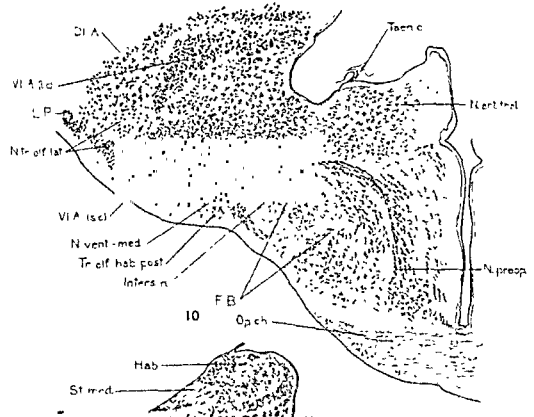


Fig. 9 Section through the level of the diagonal band of Broca, showing the relations of the parolfactory nucleus and primordium hippocampi (27 : 1, 4). (*A A'* and *B B'* show the orientation of figure 30.)

Fig. 10 Section through the anterior end of the thalamus. Note the relative positions of the ventro-lateral, small-celled area and the nucleus anterior thalami (29 : 3, 1).

Fig. 11 Section through the nucleus lateralis thalami. Note the large size of the cells (31 : 2, 3).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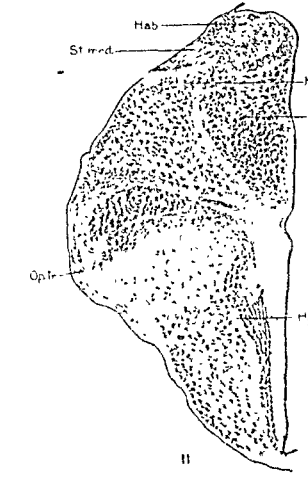
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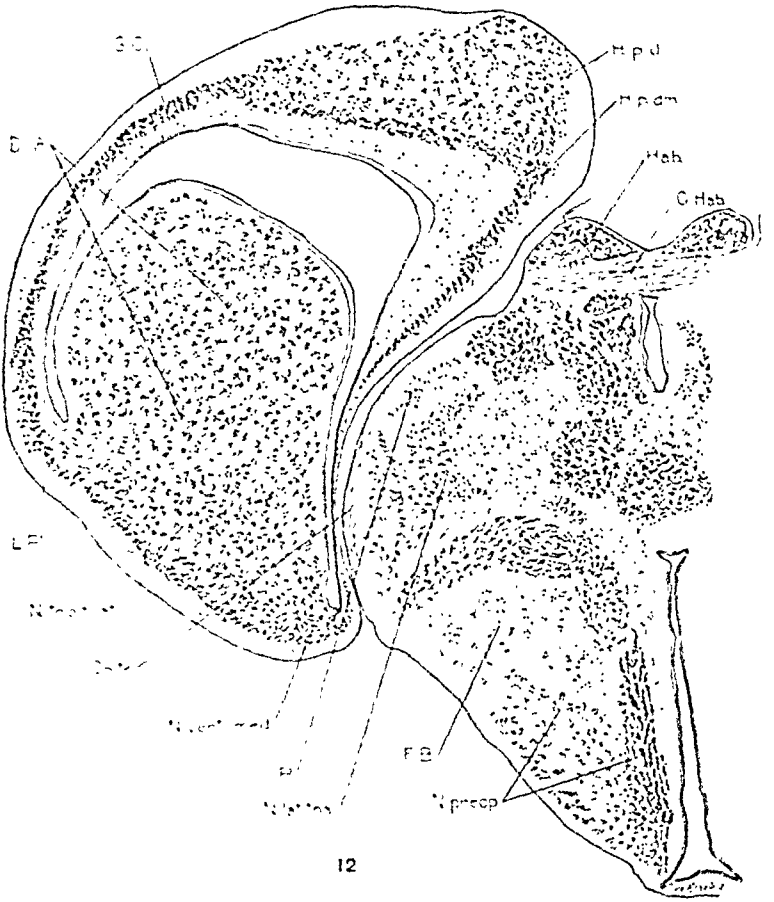


Fig. 12 Section through the habenular commissure. (32 : 3, 3).

Figs. 13–21 Transverse sections prepared by the Cajal method. Sections from two different series were used in preparing this series of drawings. ×13.

Fig. 13 Cross-section through the left olfactory bulb anterior to the olfactory ventricle. The characteristic groupings of the internal and external granule cells and the ring-like arrangement of the mitral cells are clearly shown. The incoming fila olfactoria and the glomeruli are shown in the figure (3 : 3, 2).

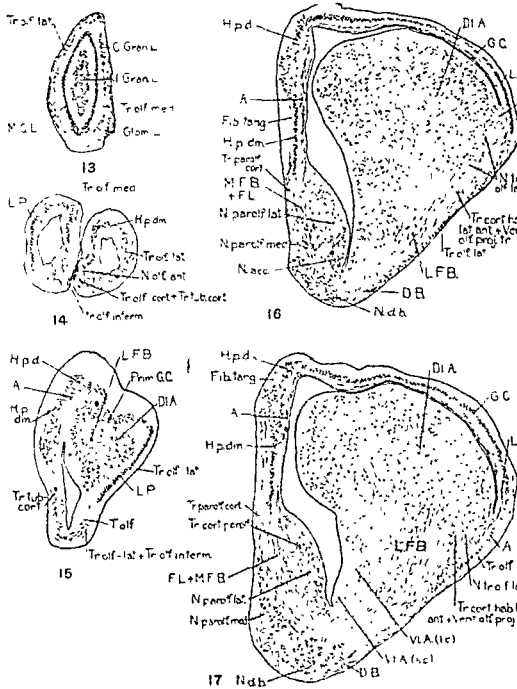
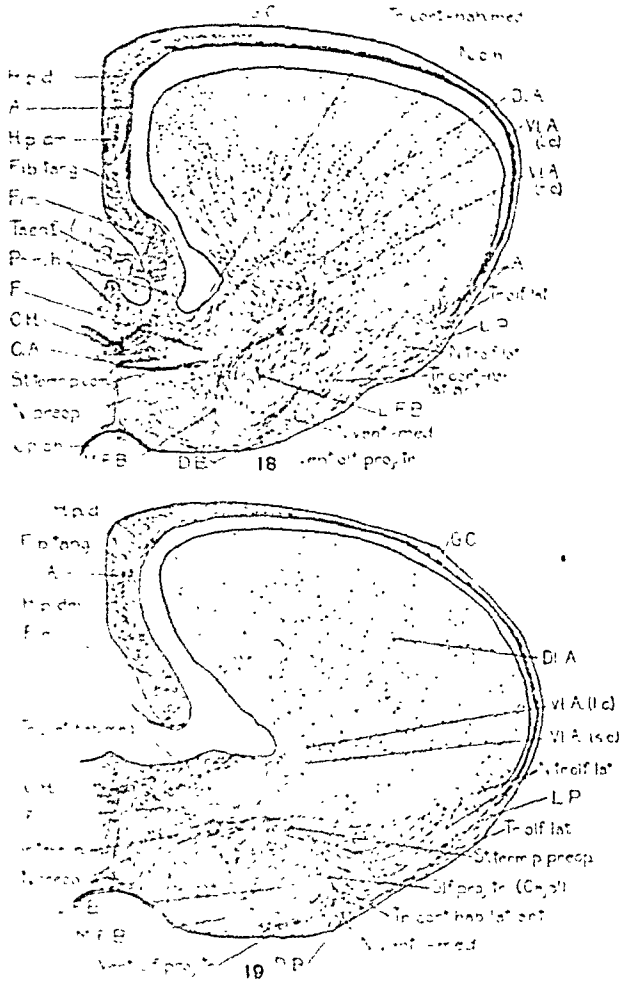


Fig. 14 A transverse section through the posterior part of the olfactory crus where it is broadening out into the hemisphere (3 : 254).

Fig. 15 A transverse section through the right hemisphere at the anterior end of the neopallial primordium (8 : 3, 3).

Fig. 16 A section near the anterior end of the medial forebrain bundle, M. F. B. (12 : 4, 1).

Fig. 17 A section a short distance anterior to the hippocampal commissure (14 : 2, 3).



Figs. 18-21. These figures were drawn from a transverse series prepared after the Cajal method and loaned by Dr. P. S. McKibben. × 13.

Fig. 18. A section through the anterior part of the hippocampal commissure (11 : 788).

Fig. 19. A section through the posterior part of the hippocampal commissure and the beginning of the stria medullaris (11 : 788).

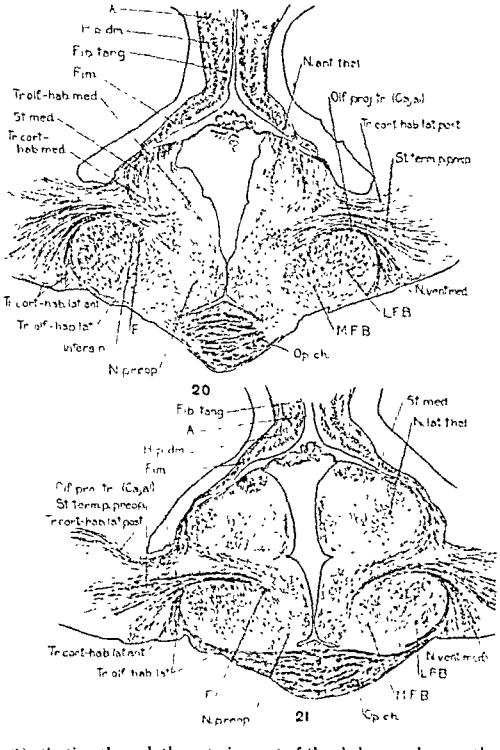


Fig. 20 Section through the anterior part of the thalamus, showing the relations of the fiber tracts (12 : 805)

Fig. 21 Section through the anterior part of the habenula (12 : 823).

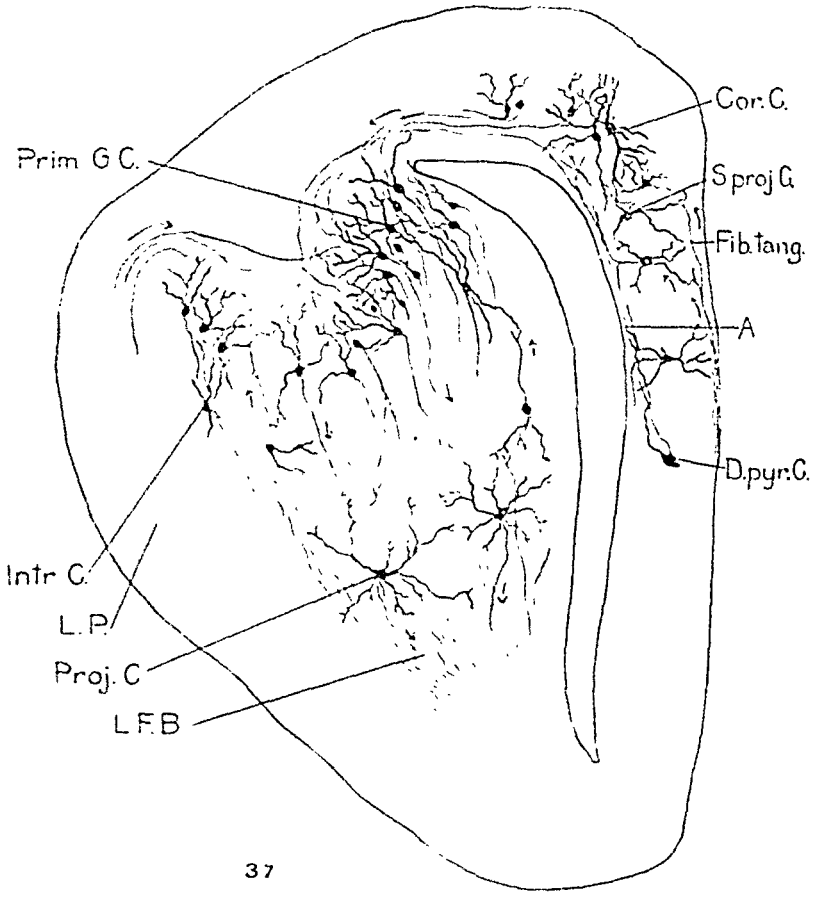


Fig. 37 This is a diagram of a transverse section through the hemisphere at the level of the primordial infolding. The cells of this primordial general cortex are round or goblet shaped (fig. 40) and have their dendrites directed outward and their axones inward and downward into the striatum. The axones come into relationship with the projection cells of the striatum, and, after a synapse, the impulse is carried by the axones of these projection cells through the lateral forebrain bundle to the lower centers. Impulses reach the primordial general cortex from the hippocampus, the pyriform lobe and the thalamus (by way of the lateral forebrain bundles). The interpolated neurone (*Intr. C.*) pictured in the diagram was not brought out very clearly in the Golgi sections, for, although neurones of that type were seen in the sections, they were never clear enough for high power drawing. Several types of neurones can be distinguished in the Golgi preparations and the cell labeled 'intrinsic cell' is a guess at one of their probable functions.

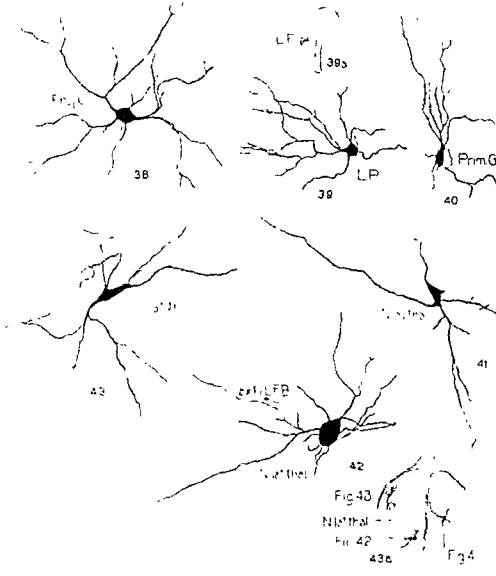


Fig. 38 Projection cell of the ventro-lateral area. For orientation see figure 37 (Gl : 126).

Fig. 39 Cell from the anterior part of the pyriform lobe (Gl : 99).

Fig. 39a Diagram for the orientation of figure 39

Fig. 40 Cell from the primordial general cortex (Gl : 105). For orientation see figure 37.

Figs. 41–43 Cells of the nucleus lateralis thalami (Gl : 160; Gl : 159; Gl : 159).

Fig. 43a Diagram for the orientation of figures 41–43.

A second test was therefore made using, in this case, an albino rat suffering from so-called 'pneumonia.' This animal had dropped from 264 to 184 grams in body weight in 66 days. Autopsy showed badly infected lungs with hemorrhagic areas and pus cavities.

A brother from the same litter was used as a control for this animal. The control animal had increased in body weight from 267 to 315 grams during the same period of 66 days. Autopsy negative.

TABLE 2

NO.	AGE	WEIGHT AT TIME OF KILLING	NUMBER OF FIBERS IN TRIGEMINAL NERVES		AVERAGE SECTIONAL AREA OF FORTY LARGEST FIBERS IN SQUARE MICRA					
					Right			Left		
			Right	Left	Fiber	Axis	Sheath	Fiber	Axis	Sheath
352♂	251 (pneumonia)	184	2210	2230	109.2	39.6	60.6	122.2	45.9	76.3
					Combined average of right and left					
					Fiber 111.2		Axis 42.7		Sheath 68.4	
353♂	251 (control)	315	2210	2296	124.9	46.8	78.1	108.8	43.1	65.7
					Combined average of right and left					
					Fiber 116.8		Axis 44.9		Sheath 71.9	

Table 2 gives the summarized records of the examinations made of both right and left nerves from both the 'pneumonia' animal and its control. In this case the technique followed was the same as in the first test. Forty of the largest fibers of each nerve were measured in this instance, giving the sectional area of the entire fiber, its axis and its sheath in square micra.

It will be seen from table 2 that in the diseased animal the average sectional area of the 40 largest fibers from the right nerve is less, while the average sectional area of the 40 largest fibers from the left nerve is greater than in the corresponding fibers of the control animal. If the averages from the right and

left nerve fibers of each animal are combined it will be seen that the fibers of the diseased animal are slightly less in area than those of the control animal. The difference, (5.6 square micra) however, is slight and probably insignificant. The axis-sheath relation in both animals is the same—38.5 per cent axis to 61.5 per cent sheath.

This test left the matter in doubt, but it seemed desirable to examine other cases of diseased animals.

'PNEUMONIA' RATS

For this further work, three groups of so-called 'pneumonia' rats were examined—each group being controlled by a healthy animal from the same litter.

TABLE 3

Series 14. Pneumonia rats and controls from same litters

Pneumonia rats

NUMBER	SEX	LITTER	AGE	STRAIN	PREVIOUS MAXIMUM WEIGHT	WEIGHT WHEN KILLED	AUTOPSY	NUMBER OF FIBERS		AVERAGE SIZE OF FORTY LARGEST FIBERS IN SQUARE MICRA					
										Right peroneal			Left peroneal		
								Right	Left	Fiber	Axis	Sheath	Fiber	Axis	Sheath
352	♂	7	251	Ext.-inbred*		184	Pneumonia	2210	2230	100 2	39 6	60 6	122.2	45 9	76 3
424	♀	20	335	Ext.-inbred	213	177	Pneumonia	2122	2000	137 3	57 2	80 1	161 2	66 7	94.4
425	♂	20	335	Ext.-inbred	305	282	Pneumonia	2145	2006	133 5	53 7	79 8	135 9	55.6	80 4
445	♂	35	454	Inbred	342	201	Pneumonia	2047	1988	128 2	53 0	75 1	119 3	47.0	71 4
447	♂	35	454	Inbred	331	292	Pneumonia	2040	1916	115 4	47 5	67 9	132.0	57 4	75 5
Averages								2119	2028	122 9	50 2	72 7	134 3	54.7	79.6
											40%	60%		40%	60%

Control rats

353	♂	7	251	Ext.-inbred		315	Neg.	2240	2296	124 9	46 8	78 1	108 8	43 1	65 7
426	♂	20	335	Ext.-inbred	355	317.5	Neg	2122	2165	139 8	52.5	87 3	130 8	52 2	78 5
446	♀	35	454	Inbred	248	208	Neg.	1973	1863	109 9	41 4	65.4	127.5	55 1	72.4
Averages								2111	2108	124 9	47 9	76.9	122 3	50 1	72 2
											38%	62%		41%	59%

* Extracted inbred albino rat.

cent osmic acid, dehydrated, embedded and sectioned in the usual manner. The stimulated nerve and its control were prepared in the same solutions, thus receiving identically the same technical treatment.

Twelve animals of the same sex and strain and of about the same ages were used in these experiments and the results are here presented in table 4.

TABLE 4

Series 12. All males. Effects (volumetric) of electrical stimulation on the peripheral nerve fibers. (Peroneal nerve.)

Number	Litter	Age	Weight	Strain	Autopsy	Number of fibers		Average size of forty largest fibers in square microns					
								Right peroneal (stimulated)			Left peroneal (intact)		
						Right	Left	Fiber	Axis	Sheath	Fiber	Axis	Sheath
350		162	203	Inbred		2253	2123	96 0	38 3	57.7	101.9	39.2	62.7
363		156	261	Inbred		2080	2143	68 5	30.2	38.3	94 5	42.8	51.7
369	11	154	243	Inbred	Neg.	2013	2090	84 3	39 2	45.1	105.8	44.7	61.1
370	11	154	215	Inbred	Neg.	2125	2093	91.3	41.6	49.7	90 7	38.9	51.8
373		150	230	Inbred	Neg.	1912	1903	107.1	46.7	60.4	95.9	37.9	58.0
374	12	150	223	Inbred	Neg.	1920	1870	94.2	38 8	55.4	99.5	41.7	57.8
375	12	150	218	Inbred	Neg.	2163	2117	99 4	44.9	54.5	95.6	43.2	52 4
376		149	236	Inbred	Neg.	2058	2038	88.3	38.6	49.7	93.6	43.9	49 7
389	16	155	251	Inbred	Neg.	2020	2122	94 9	43.6	51 3	105.7	45.4	60.3
390	16	155	230	Inbred	Neg.	2044	2066	82.1	36 3	45.8	83.6	37.2	46.4
437	33	150	248	Inbred	Neg.	2166	2150	101.8	40.4	61.4	110.6	43.5	67 1
438	33	150	237	Inbred	Neg.	2103	2113	116.2	52.2	64.0	103.3	43 4	59.9
Average		153	235			2071	2069	93 7	40 9	52.8	98.4	41 8	56.6
									44%	56%		43%	57%

The age of one animal was 162 days, all the others range between 149 and 155 days, and the group for this purpose may be considered of one age—an average of 153 days. Autopsies made on ten of these animals proved to be negative. Two were not autopsied, but showed no signs of disease. It will be noted that the average number of fibers on the right and left sides is practically identical—right 2071, left 2069—adding further proof of symmetry so far as number of fibers is concerned.

The variability in number is shown by the following determinations.

In making these determinations the formulas given by Davenport ('99) have been used for the standard deviation, σ, the coefficient of variability, C, and the probable errors of these, as well as of the mean, M.

Number of fibers in right peroneal nerve—average of 12 cases. Average age 153 days.

M (mean)	2071	$M_\epsilon = \pm$ 18
σ	96	$\sigma_\epsilon = \pm$ 13
C	4.6	$C_\epsilon = \pm$ 0.60

Number of fibers in left peroneal nerve—average of 12 cases. Average age 153 days.

M (mean)	2069	$M_\epsilon = \pm$ 17
σ	87	$\sigma_\epsilon = \pm$ 11.9
C	4.2	$C_\epsilon = \pm$ 0.51

In examining the sectional area of fibers we note that in 8 of the 12 cases the fibers of the right or stimulated nerve are less in sectional area than those of the left nerve. In one case (No. 363) the difference is large.

Taking the average sectional area of the stimulated fibers we find it to be 93.7 square micra, while the average of the left fibers is 98.4 square micra. The difference here shown between the averages is 4.7 square micra or approximately 5 per cent of the smaller number.

An examination of these measurements by the usual statistical methods gives the following results, table 5.

TABLE 5

	Right peroneal *Stimulated fibers*			Left peroneal *Control fibers*	
M (mean)	93.7 sq. micra	$M_\epsilon = \pm$ 2.3	M (mean)	98.4 sq. micra	$M_\epsilon = \pm$ 1.1
σ	11.8	$\sigma_\epsilon = \pm$ 1.6	σ	5.9	$\sigma_\epsilon = \pm$ 0.8
C	12.6	$C_\epsilon = \pm$ 1.7	C	6.0	$C_\epsilon = \pm$ 0.8

The difference obtained between the stimulated and the control fibers is 4.7 square micra. The probable error of this determination is $\pm$2.5.

The variability is shown by the following determinations:

Number of fibers in right peroneal nerve—average of 15 cases. Average age 150.9 days.

M (mean)	2038	$M_\epsilon = \pm 17$
σ	97	$\sigma_\epsilon = \pm 11.9$
C	4.7	$C_\epsilon = \pm 0.57$

Number of fibers in left peroneal nerve—average of 15 cases. Average age 150.9 days.

M (mean)	2032	$M_\epsilon = \pm 13$
σ	80	$\sigma_\epsilon = \pm 9.9$
C	3.8	$C_\epsilon = \pm 0.47$

A comparison of the average sectional areas of the 40 largest fibers of the right and left peroneal nerves shows that in four

TABLE 6

Series 15. Controls. Normal number and size of myelinated fibers in peroneal nerve

NUMBER	SEX	LITTER	AGE	STRAIN	WEIGHT	GAIN IN WEIGHT LAST FIFTY DAYS	AUTOPSY	NUMBER OF FIBERS		AVERAGE SIZE OF FORTY LARGEST FIBERS IN SQUARE MICRA					
										Right peroneal			Left peroneal		
								Right	Left	Fiber	Axis	Sheath	Fiber	Axis	Sheath
325	♂	1	150	Inbred	212	31 5		1959	1892	95.3	32.8	62.5	99.9	31.3	65.6
328	♂	2	151	Inbred	210	45 0		2279	2068	113.4	40.7	72.7	103.1	39.0	64.1
340	♂	3	152	Inbred	239	46 5	Neg.	2175	2131	98.3	36.9	61.4	97.8	36.0	61.8
342	♂	4	151	Inbred	274	69 5	Neg.	2078	2158	103.5	38.6	64.9	75.5	29.5	46.0
347	♂	6	147	Inbred	215	55 0	Neg.	2040	2018	77 4	30.0	47.4	70 0	26.5	43.5
380	♂	13	151	Inbred	256	55 5	Neg.	2123	2088	98.7	39.0	59.7	92.1	38.2	53.9
381	♂	13	151	Inbred	283	53 5	Neg.	1985	2020	101 3	43.7	57 6	95.4	38.2	57.2
384	♂	14	153	Inbred	276	33 0	Neg.	1931	1925	99.4	44 0	55.4	81.5	31.8	49.7
385	♂	14	153	Inbred	256	35 0	Neg.	1887	2085	89.7	36.8	52.9	89.5	39.0	50.5
388	♂	15	151	Inbred	238	60 0	Neg.	1967	1985	101 0	41.4	59.6	103.5	41 3	62.2
394	♂	18	150	Inbred	276	53 0	Neg.	2045	2048	100 2	40 3	59.9	97.1	37.2	59.9
396	♂	18	150	Inbred	314	78 0	Neg.	2091	2131	91 4	35.2	56.2	90.6	35 2	55.4
403	♂	21	151	Inbred	260	85 5	Neg.	1982	2003	92 2	35.4	56 8	101.0	41.9	59.1
429	♂	34	151	Inbred	233	43 0	Neg.	1989	1897	97 1	40.5	56.6	82.9	32.2	50.7
442	♂	34	151	Inbred	290	41 5	Neg.	2039	2025	92 6	41.8	50.8	92.9	37.4	55.5
Average			151					2038	2032	96.8	38.5	58.3	91.5	35.8	55.6
											40%	60%		39%	61%

cases the two sides are within 1 per cent of one another; in 8 of the remaining eleven cases the average of the 40 largest fibers is greater on the right side.

The axis sheath relation here is practically 40 per cent axis to 60 per cent sheath.

Taking the 15 cases together, the average size of fibers of the right peroneal is 96.8 square micra, the average of the left 91.5 square micra, the difference being 5.2 square micra, indicating that the largest fibers of the right peroneal nerve are more than 5 per cent greater than those of the left.

Examining these results by statistical methods we obtain the following table 7.

TABLE 7

Right peroneal fibers			*Left peroneal fibers*		
M (mean) 96 8 sq micra	$M_e = \pm 1.3$		M (mean) 91.5 sq micra	$M_e = \pm 1.6$	
σ 7 6	$\sigma_e = \pm 0.9$		σ 9.7	$\sigma_e = \pm 1.1$	
C 7 9	$C_e = \pm 0.9$		C 10.6	$C_e = \pm 1.3$	

The observed difference between the average sectional area of right peroneal fibers and left peroneal fibers as shown by table 7 is 5.2 square micra.

The probable error of this determination is ± 2.1; a little more than one-third of the difference observed.

If, however, the first seven entries of table 6 be considered separately the average of the right peroneal fibers is found to be 7.7 square micra greater than the average of the left peroneal fibers, while in the last eight entries this difference is only 3.1 square micra. If No. 342, which presents the greatest difference between the right and left fibers, be omitted, then the average of the right peroneal fibers becomes 96.4 instead of 96.8 and the average of the left peroneal fibers becomes 92.7 instead of of 91.6, and the difference becomes 3.7 instead of 5.2 square micra.

From the statistical examination of table 6 and this further analysis of its contained data we may safely assume that the difference here shown between the sectional areas of the largest fibers of the left peroneal nerve and those of the right peroneal

weight, but were not known to be of the same litter or of the same age.

Comparing male 'pneumonia' animals with male controls the total number of right and left peroneal fibers of the diseased animal is in every case less than the total number of right and left peroneal fibers of the control; this difference is 5.8 per cent.

No significant differences were observed between the sectional areas of fibers from 'pneumonia' rats and those from the controls. The axis-sheath relation in both 'pneumonia' rats and their controls is 40 per cent axis to 60 per cent sheath.

In this group of rats from 251 to 454 days of age, the older the animal the fewer the myelinated fibers found in their peroneal nerves. This applies to both 'pneumonia' and control rats. The fibers of the greatest sectional area occur in those animals 335 days of age, the younger and the older animals present fibers of less sectional area.

Data are presented to show that from age 147 days to age 251 days the peroneal nerve gradually acquires more fibers at the rate of about two fibers per day. In two groups of three animals each, aged 335 days and 454 days, respectively, the number of fibers is shown to decrease with advancing age. Between 251 and 335 days of age the rat acquires its largest and its greatest number of peroneal fibers.

In a series of twelve animals in which the right peroneal nerve of each rat was electrically stimulated for thirty minutes the sectional areas of the stimulated nerve fibers were slightly less when compared with those of the left or intact nerve, but this difference may be regarded as insignificant.

Fifteen albino rats were examined to determine the normal size of peroneal fibers of the right and left sides. This examination of the largest fibers showed that there is practical symmetry between the right and left peroneal fibers.

Twelve normal inbred albino rats of 153 days average age have an average of 2070 fibers in their peroneal nerves and the average sectional area of the ten largest fibers of these peroneal nerves is 108.6 square micra.

CONCLUSIONS

In the five series of albino rats here examined the axis-sheath relation varies but slightly. Its range is from 38 per cent axis: 62 per cent sheath to 43 per cent axis: 57 per cent sheath; an average of 40 per cent axis: 60 per cent sheath.

The symmetry as to number of fibers in the right and left peroneal nerves is almost exact, the difference shown in one group of twelve and another of fifteen animals being less than 0.3 per cent.

Pathological conditions like the so-called 'pneumonia' which is more or less acute and occurs during the later period of growth in albino rats, appears to lessen the number of myelinated peroneal fibers, but produces no measurable change upon the sectional area of the largest fibers.

In the examination of fifteen rats measurements show that symmetry as to sectional area of the largest peroneal fibers exists between the fibers of the right and left peroneal nerves.

Electrical stimulation of a peroneal nerve for thirty minutes appears to have no measurable effect upon the sectional area of nerve fibers.

In the data presented there is confirmation of the work of Dunn ('12) that sectional area of myelinated fibers decreases slightly in old age; of the work of Greenman ('13) that the intact nerve of an operated animal loses in both number and sectional area of fibers.

The number of fibers in the peroneal nerve increases with age until age 250 days is reached and begins to decrease at or before 335 days of age. After the first year of life the sectional area of peroneal fibers decreases with advancing age; at 335 days of age this process of reduction has already begun.